

A licence for Big Brother

The British government's Data Protection Bill is so illiberally drawn as to excite and not to assuage fears of intrusion into people's privacy. It should be amended radically by Parliament.

THE British government has only itself to blame that its Data Protection Bill should improbably have united in opposition the British Medical Association, the Law Society and the National Council for Civil Liberties. For the truth is that the government is and always has been lukewarm about the claim that data banks should be regulated by law so as to safeguard people's rights to personal privacy. What else can explain that it has taken more than a decade for even this halfhearted measure to see the light of day? Yet it has been plain ever since the 1960s, when the Council of Europe first persuaded its members to begin negotiating a convention on data protection, that something would have to be done. The bill that has now appeared falls far short of the advice of the committees set up to examine the question, most recently the Lindop committee reporting in 1978, and is the bare minimum required to satisfy Britain's obligations under the European Convention. The bill, published in obscurity on 22 December (HMSO 405038 £3.80) and now begun (in the House of Lords) on its parliamentary travels, also demonstrates that the government has been apparently indifferent to the torrent of constructive criticism prompted by the White Paper on the subject published last April.

The chief objective of the bill, as required by the European Convention, is that the existence of computerized data banks containing information about people should be publicly known, and that private persons should have access to data concerning themselves and also, if justified, a right to demand corrections. So far, as even the National Council for Civil Liberties acknowledges, so good. For what it is worth, British consumer legislation already requires that agencies offering advice on people's credit-worthiness should to some extent be open to interrogation. The generalization of this principle to all kinds of data banks has been made necessary by the common knowledge that new computing machinery will soon technically allow Aldous Huxley's Big Brother to exist. That something must be done is common ground. But what?

The central issue dividing the government and its critics is that the Data Protection Bill does not safeguard the right to privacy as such but merely proscribes some (not all) of the ways in which liberty may be compromised by the new computer technology. If the bill becomes law, users of data banks will have to register their existence and specify their intentions and will then be held to have committed a criminal offence if they use the data for some purpose they have not disclosed. Thus *Nature* will be required to register its use of a computer file containing the names of those who are good enough to act as professional referees of scientific papers (because the file is more than a mere list of names and addresses) and it would then be an offence to hand over the file to, say, a manufacturer wishing to promote some product at least if that intention is also undisclosed. In principle, that device should enable private persons to discover what uses are being made of data concerning them. But if the underlying objective is the protection of people's rights to privacy, should not the interdiction apply also to data banks compiled and maintained by conventional techniques? The National Council for Civil Liberties has made the substantial point that elsewhere in Europe, where several signatories of the convention have adopted liberal legislation, the majority of registered data banks are still conventional systems. The British bill, as drafted, will simply

provide malign users of data banks with an incentive to store data manually or on punched cards (not now covered).

The distinction in the bill between conventional and computerized data banks is thus both artificial and dangerous. Thus, as things are, most medical records are kept conventionally by hospitals and physicians, and so will not be covered by the bill. The British Medical Association, to its credit, is alarmed that there is nothing in the new arrangements to prevent the formal owners of these records, Regional Health Authorities and the National Health Service, from using this mass of data for the most evil purposes. No doubt, for example, there would be a ready sale to potential employers of confidential information about a person's psychiatric history. The association has stoutly taken the view that physicians should not contribute to the compilation of data banks in which privacy is not assured. The government's unwillingness to accept this view of the medical profession can only leave the impression that it has sinister intentions of its own. So broadening the scope of the bill must be one of the central objectives for Parliament in the weeks ahead.

The intended list of exceptions from the provisions of the new legislation is even more alarming. The European Convention allows that legislation may be drawn in such a way as not to compromise national security, which is obviously essential. But the British bill allows any minister of the Crown to declare his department's data banks to be in the interests of national security, and thus have them remain unregistered. Worse still, the bill exempts from the proposed controls the use of data banks by the police, tax officials and "for the control of immigration". While nobody asks that such activities should be denied the benefits of modern machinery, is it necessary that the mere existence of such data banks should be a state secret? These, after all, are precisely the fields in which people rightly fear that access by the authorities to confidential data, especially if it is false, may infringe their personal liberty. And it is intolerable that the bill should exempt from the controls the transfer of data from a private to a public data bank whenever it is decided (by the authorities) that the result will be the more effective prosecution of criminals or control of immigration. As it stands, the bill looks more like a licence for Big Brother than a restraint upon him.

As it stands, the bill is probably unworkable. The Law Society, well pleased that the bill allows that legal privilege should not be infringed by the right of access to data files, makes the cogent complaint that the definitions of the data banks to be covered by the new legislation are so loose that the first Registrar is likely to be overwhelmed by business and thus unable to concentrate on the issues that matter. On the face of things, the bill will require that users of simple information retrieval systems will have to register them, and to allow access by anybody whose name may be mentioned. Similarly, people who keep in their personal computers lists of frequently used telephone numbers will be required to register them unless they can show that they have the consent of all those whose names occur. It is curious that the government whose definition of the legal scope of its bill should have been so narrow should have allowed such a loose definition of what it wants to register. Is this some device for throwing sand in the works before they have been set up?

It is to be hoped that Parliament will be sufficiently seized of the importance of these objections to the bill — principles as well as



practicalities — that it will force amendment on an evidently unwilling government. At this stage, it may however be too late to secure what has for several years been a central issue for those concerned with the protection of privacy — the independence of the administration of legislation from the government: under the proposed arrangement, the Registrar will be responsible directly to Parliament, but the Home Secretary will have the power to change the rules under which the Registrar must operate. Similarly, the tribunal that will hear appeals against the Registrar's decisions will be controlled by the Home Secretary. This arrangement flies in the face of all the advice the government has been given in the past decade, most recently by the Lindop committee which asked that there should be an independent commission to supervise all aspects of the law. Although, given good sense all round, the new arrangement may work, it will not satisfy what should be the most obvious need — to provide the public with an assurance that the proposed legislation will assure the preservation of liberty. The new Data Protection Bill makes a number of concessions towards that objective, but they are too hedged about to be convincing.

Science for all

A call for more science and new exams in schools should be heeded.

A MEASURE of calm seems to be returning to British secondary education. That seems to be the assumption underlying this week's intelligent report from the committee set up by the Royal Society on science education in British schools (see page 276). Sir Harry Pitt and his colleagues take as given the external constraints on secondary education consisting of the funds made available by central government and the arrangements made in the past few years to rationalize the British system of public examinations. The committee seems also to have accepted the view of teachers that they need (even deserve) a respite from the rash of curriculum development in the 1960s. It is certainly prudent, but may also be wise, to work within this framework. And with these limitations, the committee has been almost daring in paying more attention to the need that all school-leavers should know what science is, and is about, than to the need to cater for the precocious few who seek to dedicate themselves to mathematics or high-energy physics soon after being weaned. To ask that a fifth of students' time in secondary education should be spent on science is, within the given framework, reasonable. If, with the passage of time, one consequence is that British public administration is less ignorant than at present of science and its application, as the committee hopes, everybody will benefit.

Observers of British secondary education, and of science education in particular, will nevertheless be disconcerted by the implications of some of the assumptions the Pitt committee has accepted. Historically, British secondary education was a device by means of which a small proportion of young people acquired a preparation for later professional life in the Church, the law or, with the help of the universities, in scholarship. With the advent of the late Lord Butler's Education Act in 1944, and of the application of the precept of further education for all a decade later, the belief nevertheless endured that secondary education is a preparation for a career. This is why so much attention has since been paid not merely to the grades that students achieve in their school-leaving examinations but to the "subjects", as they are called, in which those examinations were held. One inconsequential result is that a person with a school-leaving examination grade in mathematics is more able to find a job as a typist with a computer company, while those whose school-leaving grades are in history or Latin are more likely to finish up typing in a lawyer's office. The chances are that neither will be able to speak French or German, which are infrequently taught in British schools, and then usually badly. The folly of the 1944 act, on which British secondary education is now based, is that it decreed that secondary education should be universal but said nothing about what it should be like (except that religious study

should also be universal).

At least for the past decade, successive British governments have been trying to remedy that omission, so far without conspicuous success. Since Mrs Shirley Williams's time as Secretary of State for Education and Science seven years ago, the central British government has been trying to define a core curriculum for secondary schools and to foist it on local education authorities. The present government's consultation document on this question now seems destined to fill the gap, at least to the extent that it will tell secondary schools that their first obligations are to literacy and numeracy. But the question of what should be the components of a truly liberal secondary education has never been seriously discussed.

A return to Latin? Modern languages? Machine drawing or its modern equivalent, computer programming? The long-term weakness of the Pitt committee's argument is that it supposes these questions need not be answered, or even asked. But by refraining from rocking the boat of British secondary education, the committee has also shortened the short-term odds against having its claim on teaching time accepted.

So what place should science occupy in such a pattern of secondary schooling? Pitt and his colleagues are right to ask for what is called equal time, and right to take as their starting-point the assumption that all students need to know what science is about. They are probably, however, mistaken in their belief that a systematic shuffling of students between classrooms labelled as physics, chemistry and biology will provide a sound education for everybody. Especially because of the discovery in the past few years that students showing little interest in formal science studies have nevertheless been attracted to the field by the fun and games (sometimes, literally the games) of computer technology, the present may be a splendid opportunity for converting British students to a broader view of the importance of scientific work.

All this is uncontentious. Where the Pitt committee's proposals will meet the stiffest opposition is on the examination front. It is now accepted, as a matter of explicit policy by the British government, that there will in future be a common system of examinations at sixteen, when four-fifths of British students leave to do other things (or now, increasingly, to do nothing). So, for many leaving students, the examination at sixteen is the last chance at school to obtain an educational qualification of any kind. The inevitable consequence is that teachers advise their students to concentrate on only a few subject areas, that the rest of the curriculum is for practical purposes forgotten and that school-leavers' education is needlessly made narrow. Would it not be better, even at this late stage, to go over to some quite different system in which schools rather than formally constituted external boards would certify the attainments of their leaving students?

Similar difficulties on a far more serious scale will be encountered by the committee's proposals for reforming examinations held later in students' careers. For years it has been widely accepted in England and Wales that the present arrangements for educating young people between sixteen and eighteen are a recipe for lopsided education. People are expected to rehearse at school the studies they hope to follow at university and elsewhere in higher education. Attempts to reform this system in the past few years have however been defeated by the insistence of universities that school preparation for specialist study at universities or polytechnics is an essential means of enabling students to complete degree courses in a reasonable time. The Pitt committee now suggests that there should be public examinations at eighteen plus that would go some way to meet the needs of a broader education at the end of young people's school careers, but nobody can be sure that the universities will fall in with these liberal proposals, or that they will be acceptable to any but the most able students (who need them least). But would not a breaking of the link between the pattern of students' studies at school and at university make it necessary to lengthen university degree courses? Perhaps. But is that too high a price to pay for getting rid of a system of education whose faults are increasingly apparent?

UK academic inventions

NRDC may lose first option on patentable ideas

INVENTORS in UK universities and research institutions will have rights of control over patentable ideas arising from their work if the proposals now being considered by the government are implemented. The change is intended to be a significant stimulus to encourage the transfer of technology from the universities to industry.

The proposals, contained in an inter-departmental report, would abolish the current automatic right of the National Research and Development Corporation (NRDC) to take first refusal on patents arising from publicly funded research. Rights would go instead to the funding bodies — such as the research councils — which would then pass them on to the universities and scientists concerned. These ideas are believed to have strong support at Cabinet level (notably from Industry Secretary Patrick Jenkin and Education Secretary Sir Keith Joseph) and could be approved within weeks if the Treasury can be persuaded to agree.

NRDC has faced a lot of criticism since it was created in 1949, mainly for being too slow in evaluating ideas submitted to it (delays of more than a year have been quite common) and for being financially too conservative, putting its resources into safe money-spinners (such as the cephalosporin antibiotics) while failing to take on some more risky projects. In 1981 the Advisory Council on Applied Research and Development produced a report criticizing NRDC's role, suggesting that its monopoly over patent rights was against the public interest because of the delays it introduced and because it had failed to educate would-be innovators in the realistic appraisal of their ideas.

However, since NRDC became linked two years ago to the National Enterprise Board (in a merger not yet complete) to form the British Technology Group (BTG), a number of changes have been made which seem generally to have improved its image. NRDC at present invests its own money in selected projects, and also seeks outside support for others; officials are quick to point out that NRDC has to be prudent with its own investments because although it has a £50 million overdraft facility it is still required to show a profit each year. Although a spokesman admitted that, given the choice, BTG would probably have preferred to keep the NRDC monopoly, it is making a virtue out of necessity by saying that the proposed changes would enable it to be "more selective" in future investments.

A spokesman for the Committee of Vice-chancellors and Principals said the universities would welcome the first refusal option on promising developments, but added that BTG's services would continue to be used by both universities and research councils for handling patent applications, where it has "unparalleled expertise". BTG will also continue to play an important role in supporting capital-intensive projects, while universities will increasingly make their own arrangements with entre-

preneurial companies to develop smaller-scale ideas — exactly the sort of link that is already developing in some universities and which the government is anxious to foster. Universities would gain from royalty-sharing agreements with inventors but are unlikely to risk their own capital.

Dr John Midgley, who started the University of Edinburgh's very successful patenting and licensing scheme, is delighted with the proposals. He points out that while NRDC-supported projects in Edinburgh have made £27,000 for the university over thirty years, the patenting and licensing scheme has forged links with industry and raised some £250,000 in 10 years: he sees Edinburgh as an example of what more universities must do. The new plans should make it easier for them to do it, and may produce substantial returns for the research councils, who are starting to realize they may have been missing out.

Tim Beardsley

US high-energy physics

Budgetary shenanigans

Washington

THE Department of Energy (DoE) is trying to resolve the budgetary confusion left by Congress's hasty action last month that, in the words of the agency's high-energy physics director, threatens "complete and utter disaster".

Although the director, Dr William Wallenmeyer, says he is "optimistic that something will be worked out", a full restoration of funds to the high-energy physics programme may require Congress to take the politically difficult step of re-opening action on the 1983 budget. Such action is made even more unlikely by the congressional leadership's reluctance to reopen debate on the controversial Clinch River breeder reactor, which is part of the DoE budget.

On the other hand some members of Congress may find themselves persuaded of the vitality of particle physics in Europe and the need to bolster the US effort by the news, last week, of the probable discovery of the intermediate vector boson at the European Centre for Nuclear Research in Geneva (see page 285).

The problems began when Congress failed to pass a regular appropriation bill for most of DoE's programmes, including high-energy physics. The hastily constructed catch-all spending bill that Congress passed just before adjourning for Christmas in effect froze DoE's spending at 1982 levels, despite the Administration's request to increase funds for high-energy physics from \$364 million to \$429 million. The Administration wanted to balance this and other increases with cuts in spending on solar, conservation and fossil energy programmes.

Some adjustments appear possible without resorting to congressional action. According to a staff member of the House

of Representatives Appropriations Committee, some unspent funds from 1982 could be shifted to high-energy physics, and the multi-year financing of the national laboratories will also provide some flexibility. But these adjustments would still leave DoE's general science and research fund — of which high-energy physics consumes the lion's share — about 5 per cent short of the Administration's request. Money could not be transferred from solar energy to general science and research without the approval of Congress.

Dr Leon Letterman, director of DoE's Fermilab, said that he has had to exercise some caution in his spending as a result of the uncertainty over the budget. "We are nervous about it", he said. "I'm moving at a pace somewhere between the worst that could happen and the President's request." Letterman said that although he could recover everything he has so far spent should the worst happen, he will have to begin cutting back projects "if we don't get definitive action within a matter of weeks".

Letterman noted that "everyone realizes it was a mistake", but no-one seems to know quite what to do about it. In an ironic deviation from the usual budget tangles that federal agencies face, Congress, the Office of Management and Budget and the affected agency are all in apparent agreement that funding should be increased.

One possible solution would be for Congress to pass a so-called "no-cost supplemental" to the budget, which would simply authorize DoE to reprogramme the funds already appropriated. The Administration is expected to make a decision very shortly — probably before the President's proposed 1984 budget is announced on 31 January — on what action to seek.

Stephen Budiansky

US-Canada dispute

Acid rain settlement in sight?

Washington

THE seven-month deadlock between the United States and Canada over acid rain pollution may be breaking. Secretary of State George P. Shultz discussed the issue with Canadian foreign minister Allan MacEachen on 25 October; summary papers are now being exchanged between them. And representatives of both sides, whose last meeting ended stormily in June, plan to resume discussions in February.

One US official said he was "cautiously optimistic" that the deadlock could be broken "now that the two sides' foreign ministers have involved themselves personally" in the issue. The Canadian government, for its part, has modified the somewhat belligerent tone of its statements on the US position.

After taking office in 1981, the Reagan Administration rejected a Canadian call to reduce US sulphur dioxide emissions by 50 per cent a year, which, according to OTA, would cost as much as \$2,700 million per year and raise electricity rates for US consumers in the Eastern United States.

When, last fall, the National Academy of Sciences study essentially confirmed the Canadian view that the evidence of cause and effect was "overwhelming", the White House Office of Science and Technology Policy declared that the academy was not sufficiently unbiased to serve as a review group to the technical work of the joint working groups. It gave the job of peer review to a special group under the Office of Science and Technology Policy and headed by William Nirenberg, director of the Scripps Institution of Oceanography.

But within the US coordinating committee, as the US representatives are called, and within the US delegations to the joint working groups, there has been strong disagreement on how to interpret the evidence on acid rain. One scientist said that under the Carter Administration, the groups were encouraged to be liberal in their interpretation of the data. The new group, especially officials from the Environmental Protection Agency and the Department of Energy, are "much more conservative" in the way they extrapolate impacts from the limited data.

Acid rain has therefore become one of the Reagan Administration's most troubling science policy issues. It entails many scientific uncertainties (see accompanying story). It could cost \$2,000-2,700 million per year to reduce sulphur dioxide emissions of US utilities by half, to 8 million tons per year, if controls are implemented, according to Congress's Office of Technology Assessment (OTA). On the other hand, if controls are not implemented and it turns out that acid rain is the culprit in much environmental damage the US fishing, forestry and

agricultural industries could be hurt.

The US Administration, accused by Canada of ruining its lakes and fish, must decide when enough is known about the problem to justify a public policy decision. Says one congressional staff member who has studied the risks and benefits of controlling acid rain: "I would hate to have to decide this. That's what we elect politicians for."

Elected officials in Congress may take the initiative away from the Reagan Administration in coming months, when they take up the need to revise the Clean Air Act. The act is intended to control local air pollution and does not address the long-range transport of pollutants from high stacks across state and international boundaries. In the previous Congress, several New England congressmen sponsored amendments to the Clean Air Act that would have required clean-up by power plants — a burden that would fall most heavily on states in the midwest. But the revisions to the act were not passed.

The politics of acid rain may be different in the new Congress, however. A new study by James M. Omernik and Charles F. Powers of EPA's Corvallis laboratory suggests that surface waters in southern states,

such as Louisiana, Mississippi and Alabama, may be affected adversely by acid rain as well. So powerful midwestern Senators, such as Richard G. Lugar (Republican, Indiana) may have to fight a broader group favouring controls.

On 5 January, the State Department told Canada why the Administration feels that the scientific uncertainties are too great to warrant regulatory action now in the United States. The department is expecting a paper from the Canadian government explaining that country's position that enough is known to warrant interim and long-term measures. The papers are expected to be the basis for further talks between Shultz and MacEachen.

When it took office, the Reagan Administration reversed the Carter Administration's position that some kind of joint US-Canadian action might be necessary soon, and that joint studies should be made as quickly as possible to determine what that action should be.

In a joint statement in July 1979, the Carter Administration and the Canadian government agreed that some kind of environmental agreement should be sought on acid rain. In a memorandum of intent signed in August 1980, the two governments agreed to work towards such an agreement, to discuss interim measures, and to set up joint working groups to figure out the issues.

Deborah Shapley

Three points of contention

THE Reagan Administration's position is that scientific uncertainty over three major issues makes any new restrictions on power-plant emissions unjustified:

- Transformation of sulphur dioxide (SO_2) and nitrogen oxides (NO_x) to acids. The complete chemical pathway of these transformations in the atmosphere has yet to be worked out, and variations in the acidity of rain over time and space are not well explained by current theory. Some evidence suggests that the limiting factor in the oxidation of SO_2 and NO_x to sulphuric and nitric acids may be the availability of atmospheric oxidants, such as ozone and peroxide. "Conceivably", said Courtney Riordan, assistant administrator for research at the Environmental Protection Agency (EPA), "you could have a significant reduction in emissions and see no improvement in deposition as a result".

- Transport and distribution of SO_2 and NO_x in the atmosphere. Not enough is known about either long-range or local transport of acid-rain precursors to refine models to the point where a specific power-plant's emissions may be linked to acid rain at a specific downwind site.

- Natural acidity of rain. "There is no good measure of when acidity in rain should be considered detrimental", said EPA assistant administrator Kathleen Bennett. Unpolluted rain may have a pH above or below pH 5.6, the theoretical natural level based on equilibrium with

atmospheric CO_2 .

The Administration has put itself at odds not only with Canada but also with its own National Academy of Sciences in taking this line. "Although claims have been made that direct evidence linking power-plant emissions to the production of acid rain is inconclusive", an academy panel said in 1981, "we find the circumstantial evidence for their role overwhelming".

And both the academy and the Canadian government maintain that the goal of being able to link specific sources with specific environmental damage is impossible. George Rejhon of the Canadian Embassy in Washington said, "When you start working towards 'scientific certainty' you can go on forever. There are certainly some people in EPA who seem to be demanding such a degree of scientific precision that they want the natural world to be like a man-made laboratory. That is not possible."

Rejhon said the Canadian view is that enough is already known to take action on limiting SO_2 emission from power plants. He said that although it may not be possible to say how much a given region will benefit from a given reduction in emissions, the evidence is that at least 90 per cent of the acidity in areas of concern is man-made. Thus reductions in overall atmospheric loading of SO_2 will produce overall benefits in the affected areas.

Stephen Budiansky

West German ecology

Ozone named as culprit

GERMAN forests, long admired as examples of sustained high yields through careful management, have in recent years suffered degeneration that has become a matter of national concern. Since 1976, highly visible damage to spruce (*Picea abies*) has been seen in the Black Forest and in Bavaria. Similar symptoms have occurred in fir (*Abies alba*) and last year damage was found elsewhere in West Germany and in other species, including broadleaves.

Typically, with fir, the needles become yellowish and fall off. Those below the crown are first affected, causing the tree to assume a "stork's nest" appearance. Damage is most frequent to trees exposed to sunlight and wind on south-west slopes more than 800 m above sea level.

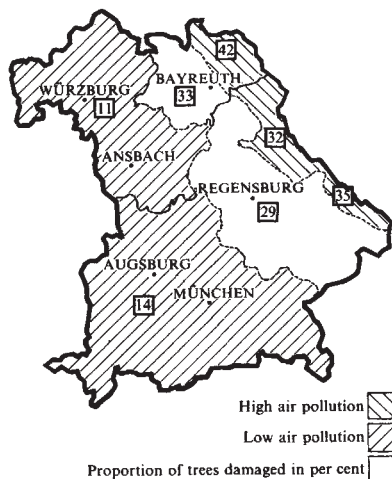
A widely held view is that the yellowing and die-back are effects of "acid rain". Dr J. Bauch of Hamburg University has proposed that the increased acidity of the soil inhibits rootlet and mycorrhiza development.

In September 1982, however, the Pollution Control Establishment in the Land of Nordrhein-Westfalia began an intensive investigation. An interim report published at the end of December concluded that the real culprit is ozone, formed by the action of light on oxides of nitrogen in the atmosphere. An impressive amount of circumstantial evidence is presented, such as the appearance of symptoms after the sunny summer of 1976 and the correlation of damage with ozone content in the atmosphere rather than with the acidity of the precipitation. And in laboratory tests, the difference in the effect of rain of pH 4.5 and 3 was slight, but exposure of spruce to ozone at 100–400 $\mu\text{g m}^{-3}$ for 3–4 weeks produced chlorotic mottling. The lichens *Hypogymnia physodes* and *Pseudoverna furfuracea* appear to be more sensitive to acid rain but less so to ozone than trees.

The proportion of affected trees varies considerably. In parts of Bavaria bordering

Czechoslovakia and East Germany (GDR), more than 40 per cent of the trees are damaged, and nowhere in Bavaria is the proportion less than 10 per cent. It was estimated last year that 7.7 per cent of trees in all West German forests had some degree of damage.

So, inevitably, pollution control has become a political issue; indeed, it was a contributory factor in the break-up of the former coalition SPD-FDP government



Damage to forests in Bavaria in 1981

last October. Herr Baum, then FDP interior minister, had been pressing for more stringent control of industrial emissions, but was opposed by Herr Lambsdorff, finance minister, also of FDP. Lambsdorff has now found a place in the new CDU-FDP coalition where he is supported by Herr Franz-Josef Strauss, the Bavarian CSU leader, who has tried to play down the seriousness of the situation despite the signs of damage in his own Land.

In reducing pollutant levels, West Germany is not its own master. About half the pollution comes from outside, chiefly from East Germany, where about 270 million tonnes of brown coal (70 per cent of total energy) was burned in 1982, and France. West Germany itself is a substantial exporter of pollution, chiefly industrial waste gases given out by tall chimneys, but by 1990 most power stations should be equipped with gas scrubbers.

Internal factors have inhibited the development of an overall environmental protection policy. There is no federal environment ministry, a situation which SPD is likely to remedy if returned to power in the March elections, and even some of the Länder divide responsibility between the interior and agriculture departments. The fact that the LIS, in central-northern Germany, recently produced a report largely concerned with damage to forests in the southern Länder of Bavaria and Baden-Württemberg, shows that inter-Land barriers are being overcome.

J.S. Dunnett

Infrared astronomy

Satellite launch of telescope

If all went well, the Infrared Astronomical Satellite (IRAS) will have been launched on a Thor-Delta rocket from Vandenberg Air Force Base in California at 0220 GMT on Wednesday, 26 January. Once launched, IRAS will embark on the first sky survey at infrared wavelengths from outside the Earth's atmosphere.

The experiment is a joint venture by the United States, which supplied the main infrared telescope and the launch vehicle, the United Kingdom, which will be controlling the satellite and receiving and processing raw data at the Rutherford-Appleton Laboratory in Oxfordshire and the Netherlands, which supplied the satellite vehicle and cooling system, together with a subsidiary infrared telescope.

The detectors are housed in a cooling vessel whose hollow walls are filled with liquid helium at 2 K, minimizing thermal infrared noise from the detectors' environment. Previous space-borne infrared detectors have been cooled to about 12 K. IRAS will provide about a ninety-fold increase in signal-to-noise ratio over the earlier experiments.

Unfortunately, the lifetime of the liquid helium is limited due to vaporization, so IRAS is likely to be useful for no longer than seven months. For almost all of that time the main telescope will be devoted to a sky survey at four wavelengths: 8.5 to 15, 19 to 30, 40 to 80 and 83 to 119 micrometres. The additional experiment supplied by the Netherlands will be used to examine particular regions of interest with greater spatial and spectral resolution than can be achieved by the survey telescope. But the survey will be interrupted only if particularly interesting objects are observed. Data will be received twice daily at the Rutherford-Appleton Laboratory and, after preliminary processing, will be distributed to observatories around the world*.

Infrared astronomy from the ground is hindered by the presence of absorbing gases (water vapour, for instance) in the atmosphere. However, peering through "windows" in the absorption spectrum over the past few decades, astronomers have detected objects such as very young and very old stars whose main signatures are at infrared wavelengths. Infrared waves can also emanate from regions shielded by gas at optical wavelengths, such as the centre of our Galaxy. As well as such expected infrared sources (about a million in all), astronomers are anticipating discoveries of new types of objects.

Philip Campbell

*A summary of the data will be published in *Nature* every two weeks during the lifetime of the satellite.



Cancer research in France

Forces for change needed

CANCER research in France is a mess of conflicting interests and isolated groups, dominated by molecular biology and with almost no interest paid to clinical research and epidemiology — this is the picture that seems to emerge from a series of colloquia just completed in France on the research and treatment of cancer.

What's to be done? After a great deal of noise, now there is silence. The President of France, M. Mitterrand, and the health minister M. Jack Ralite are to appear on television to discuss the issues. But the real action is occurring behind the scenes, in the Groupe de Réflexion sur le Cancer.

Ralite established both this body and the national colloquia (the Concertation sur le Cancer) that followed, on the advice of Dr Jean-Claude Salomon, a well-known cancer immunologist whose views are anti-reductionist, pro-patient and socialist. Salomon hoped to use the colloquia to mobilize forces for change; and now he and his Groupe de Réflexion are about to assess the results and prepare political recommendations for the minister.

Naturally enough, Salomon will say little at this stage — he would not want to alert the forces he challenges. Ranged "against" him, suspicious of reform, are the two big private cancer research institutions, the ADRC at Villejuif and the Ligue Nationale, which between them spend more on cancer research than all public bodies put together, but fail to coordinate their research policies (attempts to harmonize them were recently abandoned). There are also the specialist cancer treatment centres, the public hospitals and the private clinics (mostly owned by banks) which compete among themselves for patients.

Of the 20 cancer centres, 10 are dominated by radiotherapists, who — Salomon has said in a less guarded moment — are too powerful in France. Private clinics are also mostly devoted to radiotherapy and Salomon believes that France uses radiotherapy more than any other country.

"Why do cancer patients die?" Salomon asks. Generally not directly of the tumour, he answers, but from secondary infection or endocrine disorders. "Cancer is a disease; the tumour a part of it". Such concepts will no doubt be influencing the recommendations of Salomon's Groupe de Réflexion in the next few weeks.

The national medical research council, INSERM, and the basic research council, CNRS, are not equipped to support such interdisciplinary and integrated research, Salomon believes; instead there should be a national institution to coordinate health and research policy in relation to cancer.

Robert Walgate

French Académie

New blood, third class

FRANCE'S Académie des Sciences, the close equivalent of the British Royal Society (both were founded in the mid-seventeenth century and have similar constitutions), is to be given a fresh breath of life — by one of the liveliest 75-year-olds in France.

Professor Jean Bernard, a renowned haematologist from the Hôpital St Louis in Paris, has been elected president of the Académie, and he promises to "appeal to the youngest researchers".

Already working at 8 a.m. last Saturday morning, he said on the telephone that the Académie's greatest problem was its ageing membership. "A brilliant mathematician elected at the age of 35 will be in the Académie for 50 years", he said.

His solution is to increase the number of "corresponding members", a kind of third class of membership which does not confer voting rights but does allow participation in the activities of the Académie — which increasingly involve advice to government. This would rejuvenate the Académie without committing it to vast and irreversible expansion, Bernard believes.

Although his appointment was approved by the minister for research and technology, M. Jean-Pierre Chevènement, Bernard insists that the Académie is "entirely independent".

In his inaugural speech recently, Bernard

spoke of three distinct historical periods of relations between the government and the Académie. "First, a period of frequent consultations, from the hydraulic engineering for the gardens of Versailles to poison gas in 1915; a desert, silence for sixty years, from the poison gas to Three Mile Island [when the government again asked for advice]; then the present period, when our advice is wanted again."

Asked if he was in sympathy with Chevènement's approach to science and technology in France, Bernard replied last week that there were two dangers facing science: that it should be too concerned with the fundamental and ignore applications; and vice versa. "The Académie is largely for basic science", said Bernard. But it will not ignore applications and recently set up CADAS, the committee for the applications of science, under the chairmanship of space scientist M. Hubert Curien.

The Académie also wishes to play a role in one of M. Chevènement's favourite projects: a great French encyclopaedia of science and technology, in the tradition of Diderot, but using modern information techniques. Bernard would also like to set up interdisciplinary studies with the other partners in the Institut de France.

Robert Walgate

UK science education

Royal Society call for new exams

MODEST but practicable proposals for the reform of science education in British schools were put forward earlier this week by a committee of the Royal Society under Sir Harry Pitt, previously vice-chancellor at the University of Reading. Among other things, the committee recommends that science should occupy 20 per cent of the secondary school curriculum (with mathematics extra) and that courses and examinations should be modified so that students do not have to abandon particular science subjects. On the vexed question of specialization in the sixth form, the committee advocates science courses with roughly half the content of the advanced-level courses now followed by some 20 per cent of school-leavers.

The committee's report, "Science Education 11-18", is in part based on a specially commissioned survey of the practice of science education in state-supported secondary schools. The past opinion of Royal Society committees that schools should give special attention to the needs of students with an early and exclusive interest in science subjects is conspicuous by its absence.

The committee also makes a series of intelligent asides on questions such as the place of history in science courses and the

need that science and technology should be more conspicuous in the history taught in secondary schools.

The case for the committee's demand for 20 per cent of the school curriculum, at least in the final two years of the usual five-year course, stems from its belief that all secondary students should learn something of the whole of science and that, in most British schools, teachers are better prepared to teach physics, chemistry and biology than some form of integrated science.

On examinations, a well-known British preoccupation, the committee asks for a reduction of the content of the syllabuses on which public examinations taken at 16 are based, together with an interlocking system of examinations intended for students of different ability, a development originally suggested two years ago in connection with mathematics so as to avoid the present circumstances in which the median performance tends to qualify only for the lowest or next-to-lowest grade. Almost nostalgically, the committee notes that its proposal that there should be examinations to be taken at 18 requiring only half as much preparation as for the existing advanced-level examinations, entails a return to a system abolished in England and Wales decades ago. [1]

US research grants

NSF takes a back seat*Washington*

FROM 1 March, US universities will be granted significantly greater responsibility for the administration of research grants from the National Science Foundation (NSF). Current procedures which require that researchers obtain approval directly from NSF for such expenditures as foreign travel and purchase of permanent equipment will be replaced by a system of local review by university officials.

The new system takes a step away from efforts of past years to require more detailed cost accounting by universities and their researchers. A particular bone of contention between universities and federal funding agencies has been the widespread, but technically illegal, practice of "borrowing" funds from one grant to help pay for a second project which, for example, might be awaiting approval. Government auditors have often disallowed such charges and, more generally, have disapproved the combining of funds that one researcher may have from two or more different grants.

NSF will now, however, allow the universities to grant early starts — of up to 90 days — on projects awaiting approval (although the universities will be responsible for expenses during that period if approval is not given) and to allow the combining of separate grants to a single principal investigator if the universities determine that the projects are sufficiently related. The universities may also grant six-month no-cost extensions to NSF-supported projects.

Although the privilege of combining grants will for now be limited to those from NSF, the foundation hopes that other agencies will follow suit. But NSF says that it still wants to review requests for merging grants when two different principal investigators are involved.

NSF is dropping a proposal that all grants to a single department be combined into one master grant. According to Gaylord Ellis, director of NSF's division of grants and contracts, this scheme — which was tried out in the chemistry departments of nine universities — proved to carry too much of a bookkeeping burden for the universities.

The universities will have considerable leeway in the administrative details of the new system; NSF requires only that records be kept of all actions and that the budget decisions be passed on by at least one finan-

cially-responsible university officer and one department chairman or the university's director of research (who could be the same person).

Ellis pointed out that the change is in keeping with the current Administration's thinking on reducing federal interference, and, since "most of the requests had been approved anyway", NSF is actually giving up relatively little in way of control over its funds.

Stephen Budiansky

US university re-equipment

Long queue for DoD cash*Washington*

THE US Department of Defense (DoD), which had allocated \$30 million a year for five years to help US universities to re-equip themselves, has been flooded with requests totalling an estimated \$640 million for the first year alone.

Research managers at the Office of Naval Research, which is coordinating the programme for DoD, are shaking their heads about the inadequacy of the funds available. "We will be able to fund only one proposal out of every 21 or 22", says Robert Ryan, a staff assistant there.

By Ryan's count, 2,465 proposals were received. They had to state why the university needed the equipment and how the equipment could be used in defence-related research. Ryan gave up counting at proposal number 987, but the requests averaged \$267,000 apiece.

The \$670 million figure is significant, for it represents only instrumentation that the universities feel relates to their present or prospective defence work. Thus the real estimate of overall university equipment needs may be at least double that figure.

Universities and scientific leaders have complained for years that their equipment is becoming obsolete and that constrained federal budgets are not allowing them to replace the expensive items. This year's "DoD-University Research Instrumentation Program" is one of the very few government efforts to remedy the problem. But even Reagan's defence budgeteers — normally generous — seem to have underestimated the need.

For example, last summer a group convened by the National Research Council concluded that as long ago as 1970 there was a need for an investment of \$200 million to re-equip US laboratories. "The accumulated need is now at least \$1,000 million", it said. An earlier \$75 million once earmarked in the budget was eliminated by budget cutters. So DoD's paltry \$30 million may not be enough, but it seems the only hope in sight.

Deborah Shapley

Lead in petrol

EEC moves for complete ban

THE large group of British Conservatives (European Democrats) in the European Parliament are now putting forward a resolution for an EEC directive banning lead in petrol altogether. In this they draw considerable support from an EEC research project which has just published its interim report.

The experiment, whose sponsors include Agip, the Italian petrochemical company, and two of the major producers of lead additives, the Societa Italiana Additivi Carburanti and the Ethyl Corporation, was carried out in Turin by the Joint Research Centre at Ispra in Italy, and sought to establish just how much lead found in the blood of people living in urban areas originated in the lead anti-knocking additives used in petrol.

Between 1977 and 1979, 90 per cent of the petrol sold in Turin was mixed with lead containing lead isotopes 206 and 207. Cross-sections of the population were then sampled at regular intervals and their blood was analysed at Ispra using mass spectroscopic techniques. An interim report of the analysis has just revealed that more than 29 per cent of the lead found in the samples came directly from inhaled petrol exhaust fumes.

The experiment has yet to reveal how much lead that finds its way by other routes, such as in food and water, can eventually contribute to the total. Another EEC-sponsored experiment, undertaken in Belgium by Professor Picciotto of the Université Libre de Bruxelles, suggests that this could be as much as 50 per cent. Using a much smaller sample but also employing mass spectroscopy for tracing the origin of the lead, he concluded that petrol was responsible for between 45 and 55 per cent.

Some doubt has been cast on the value of the Turin experiment's results, however. It appears that people living in the countryside outside Turin, where there is considerably less lead in the air, were found to have just as much lead in their blood, although less of it came from petrol. One possible explanation is that the lead comes from wine, either from lead arsenate used as a fungicide in the local vineyards or from lead caps on wine bottles. For this to hold true, though, the farmers of Piedmont must be assumed to be drinking three times as much wine as the Fiat factory workers in Turin.

Also unexplained is the finding that the lead was more readily absorbed by smokers and drinkers and that towards the end of the experiment the proportion of "labelled" lead began to decrease. This may have been related to the fact that lead accumulated in the bone naturally exchanges with lead in the blood.

Jasper Becker**NSF departures**

In the news item "Carter appointees leave NSF" in *Nature* of 16 December 1982 (300, 567), Dr Eloise Clark, deputy director of NSF for biological, behavioural and social sciences, was incorrectly referred to as a Carter appointee. In fact Dr Clark was appointed by former President Ford.

X-ray satellite

Odds on Thor

AT a "restricted session" of its council last week, the European Space Agency (ESA) decided to lay the groundwork for a possible launch of the European X-ray astronomy satellite, EXOSAT, on a Thor-Delta rocket from the US National Aeronautics and Space Administration (NASA), in case the present ground trials on Ariane lead to further delays in the European launching programme. A final decision as to whether the satellite is to be launched on the Ariane or on a Thor will be made on 23 February.

ESA has been under pressure from European astronomers to take the politically embarrassing step of a Thor launch ever since it became clear that delays in the Ariane programme (after the failure of L5, the fifth Ariane launch in September 1982) were likely to harm EXOSAT's scientific potential (see *Nature* 6 January, p.8). Last week's decision represents a formal acknowledgement that the problem exists and should be catered for. As a result, scientists from ESTEC, ESA's technical centre in the Netherlands, are visiting the United States to make contingency plans with NASA. The modifications required on the satellite to switch launchers would be minor.

The key question for ESA is whether there is time to launch EXOSAT before the end of the summer launch "window" for Ariane. EXOSAT is set to be carried by the seventh Ariane, L7, but before that L6 has to be launched. It is scheduled for May but is still undergoing trials of its turbo-pumps which have been modified to take account of the failings of L5. Although everybody is tight-lipped there are rumours that the launch of L6 may be delayed until June, which would barely leave time for L7 to go up within the summer launch "window", given the expected turn around of about eight weeks for successive Ariane launches. If the rumours are true, therefore, a Thor-Delta launch is virtually a certainty — and would take place from Vandenberg Air Force Base, California, in late June.

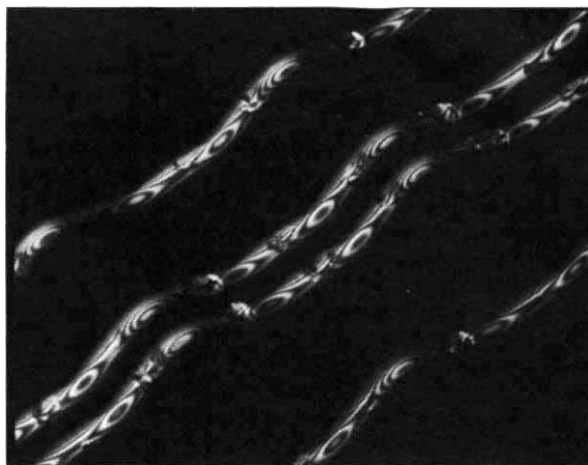
NASA will require notice for such a launch by the end of February. The financial consequences of such a decision by ESA are expected to be minor, but would depend on negotiations between ESA on the one hand and NASA and Arianespace on the other. One suggestion is that the money already paid for the L7 launch could be banked by Arianespace as a future contribution to the cost of launching Giotto, ESA's mission to Halley's comet.

Despite the political embarrassment of such a move, it could have a compensatory benefit for Arianespace. There is a queue of communication satellites waiting for an Ariane launch, and Arianespace may well be relieved of pressure and uncertainty if EXOSAT is removed from its waiting list.

Philip Campbell

Instant winner

Winner of the black-and-white light microscopy category in Polaroid Corporation's first worldwide instant photomicrography competition. The photograph is an enlargement of a polyester monofilament recorded in polarized monochromatic light on Polaroid Type 55 positive/negative film with a Carl Zeiss Ultraphot II microscope. It was taken by Karl Nettelstroth, a microscopist at Hoechst AG in Frankfurt.



Romania/US relations

Problems on both sides

WESTERN countries are attempting to employ "neocolonial" methods by recruiting academic skilled labour from Romania, a leading Bucharest daily newspaper, *Romania Libera*, alleged last week.

The claim, in an article which ostensibly reviewed the state of education in Romania, came only a few days after the return to the United States of Mr Lawrence Eagleburger, a senior State Department official who had visited Romania as a presidential envoy. A major part of Mr Eagleburger's mission was to explain to the Romanians the United States' attitude to the new Romanian emigration law which was suddenly promulgated last November. Under this law, every would-be emigrant would have to repay the Romanian state the total cost of his or her higher education, from the last two years of secondary school upwards. This sum, which could amount to as much as \$50,000, must be paid in "hard currency" — which Romanian citizens are forbidden to possess.

According to *Romania Libera* these measures have been introduced out of a "deeply patriotic concern for the patrimony of the Romanian people",

which, it says, has been exploited under various pretexts in the past. In fact, emigration is already virtually restricted to the 3,000 Jews and 12,000 ethnic Germans who in recent years have been allowed to leave annually for Israel or West Germany. The new law, if implemented, would reduce this annual flow to those few persons fortunate enough to have friends or relatives abroad to buy them out. Under Clause 504A of the United States Trade Act of 1974 (the "Jackson amendment"), the imposition of any such "education tax" on emigrants automatically excludes the country concerned from enjoying most favoured nation status (MFN) with the United States.

Should the new rule be implemented, the US Senate would be unable to renew Romania's MFN when it expires next June.

The announcement of the education tax last November drew an immediate response from the US State Department, deploring this move and drawing attention to Clause 504A. The strength of the American reaction — coupled with equally sharp criticism from West Germany and Israel — seems to have taken the Romanians by surprise. Nevertheless, during Mr Eagleburger's visit they continued to maintain that emigration is causing a serious "brain drain" and so it is only fair that the emigrants should repay the Romanian economy for its educational investment in them. This, however, does not explain why the emigrants cannot pay in Romanian currency.

So far no cases of emigrants having to repay education costs have been substantiated. The delay in implementing the law had raised hopes in the West that while not losing international face by formally abrogating the law, the Romanians might be intending to let it remain a dead letter. The tone of the *Romania Libera* article, and its timing so soon after Mr Eagleburger's visit, now make the fulfilment of such hopes unlikely.

Vera Rich



Leading question

SIR — The unsigned leading article on the editorial page is the traditional and time-honoured vehicle for the expression of the views of the Editor on matters of moment in Nature, as in other journals. News and Views, however, have always been accompanied by their authors' names — and more recently positions. It is, therefore, with some regret that I have read the anonymous full page "leaders" (?) which have introduced this section several times over the past few months, without being able to judge the bias of their originators.

That is not the case in the article "Disputes about the Earth's interior" in Nature of 23/30 December 1982 (300, 681) where the opinions aired are even more dogmatically and condescendingly expressed than is usual even in examples of this genre. Anyone prepared to express himself (themselves?) quite so contentiously should at least have the grace not to hide his name behind a masthead.

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LEADING articles, wherever they occur, are but opinionated reporting. Their function is not to be normative (pace the sociologists) but to inform, sometimes to entertain and to stimulate (sometimes by provoking). Anonymity is made necessary by the principle that while news is sacred, opinion is free and may even be that of editors, who must thus personally take responsibility. — Editor, Nature.

Armchair mutations

SIR — I can't help wondering whether the writer of "More speculation about oncogenes" (Nature 18 November, p.213) was pulling our legs. In the first paragraph we are certainly warned that "those of an austere temperament should turn the page". The article discusses the problems arising from the discovery that the difference between a normal human gene and its equivalent in a human cancer cell is a single point mutation.

I was intrigued to note that the article itself contained two point mutations and one deletion: namely "inknow" and "connot", point mutations of U and A; and "strenthened" clearly a G deletion. The relevant human gene discussed codes a 21,000 dalton protein; in other words, some 200 amino acids and 600 bases are involved. The article contains approximately 6,000 letters, enough "bases" for a 10 times larger protein, but with only one "mutation" per 2,000 bases. In spite of this low rate, I was surprised to find myself irritated and automatically corrected the errors. Does this behaviour go some way towards explaining the paradox alluded to by the writer?

ROGER PARISH

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Stereo viewing simplified

SIR — The increasingly common use of stereo diagrams to illustrate the structure of proteins and other macromolecules poses for readers the problem of superimposing the images. In my experience many have difficulty with the simple devices constructed from two lenses or two prisms. I have observed recently that most people with normal vision can obtain stereo images using a simple reading lens. The stereo pair is observed with both eyes through a single lens (3-inch diameter, or better 4 inches x 3 inches) preferably without spectacles. As the lens is moved from the diagram towards the observer one of the images will be seen to move across and superimpose upon the other. Once they are locked together, the stereo image may be focused at a convenient magnification. The method works equally well with stereo pairs of micrographs. The effect of the lens is to decrease the convergence of the eyes required to fuse the images. The only disadvantage is that the right eye observes the left image and vice versa, so that with the normal printing convention the stereo effect is inverted, the back becoming the front. For cursory inspection this does not matter, but for detailed observation the diagrams should be interchanged (using a photocopy!).

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U cells contain contaminants

SIR — Four sublines of the original U cell line have been found to be HeLa cell contaminants. The U cell line was established as a normal human amnion line by the late Dr H.J. Doorshoet in Utrecht, The Netherlands, in 1957¹. One subline was obtained from Bilthoven (I), two sublines from Helsinki (II, III) and one from a laboratory in New York City (IV).

Cytogenetic analysis demonstrated five marker chromosomes characteristic of HeLa cells² in U cell sublines I, II and III. Four of these markers were also present in subline IV. Additionally, two marker chromosomes not previously reported in HeLa cells were observed in all four sublines. One marker was probably derived from a deletion of the short arm of chromosome 7 del (p11-7pter); the other represents a translocation between the long arms of chromosomes 4 and 11.

Electrophoretic analysis of ten polymorphic enzymes supported the identity of the four sublines as HeLa with the following concordant phenotypes: G6PD A, PGM1 1, GLO1 2, ACP1 AB, ADA 1, AK1 1, PGM3 1, PGP 1, ME2 1-2 and ESD 1. An exceptionally rare observation in subline II was the ESD phenotype tenta-

EEC and agriculture

SIR — I read with interest the news item from Brussels ("Science to rescue EEC politics?", 16 December 1982, p.567) concerning Viscount Davignon's initiative at the European Commission to utilize scientific research as a tool for dealing with the high cost of the EEC's agricultural policy. Current systems of production subsidy, wherever they occur, usually strive to maximize gross output at the farm gate by absorbing direct expenses, however large, if they are sufficiently widespread amongst producers.

A system focused on increasing farmers' net income by lowering costs, even on a smaller volume of production, would have value in any political arrangement. This is most likely to be achieved in the long run by investing in biological research. A number of promising developments in the plant sciences suggest the feasibility of using genetic mechanisms to displace a significant percentage of capital-intensive inputs, such as agricultural chemicals. In addition, externalities in conservation and toxicity might also benefit. Research and development are logical tools of political economy and plant molecular biology could have profound implications for agricultural policy in its international as well as its European dimension.

DAVID PADWA

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tively typed as ESD "1-3" having one isozyme of the same mobility as ESD 1 and two slower migrating isozymes³. The number 3 allele possibly arose by mutation and selection in tissue culture.

The mistaken use of the U cell line as a normal human amnion line may not be critical in some studies, such as its term use in Finland in interferon assays, but would be in others, such as its recent use as a model normal human epithelial line unusually susceptible to Epstein-Barr virus infection⁴.

Contamination by HeLa or other cell lines is prevalent enough so that genetic analysis should be routinely done before important studies and publication of papers involving cell lines.

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Science in China

Planting a tall tree

A correspondent with wide experience of Chinese affairs describes the state of science in China in the wake of the Cultural Revolution and speculates on the possibilities for the future.

WHEN British Prime Minister Mrs Thatcher drank tea at the Shanghai Institute of Biochemistry on 25 September 1981, she became the first major foreign leader ever to visit a Chinese basic research establishment. Her call reflected not only the high priority now attached to science within China, but also China's rising profile in the world scientific community and the growing eagerness of Chinese researchers to participate in international scientific discourse.

After the chaos of the Cultural Revolution, the overwhelming impression of China nowadays is the dramatic progress achieved both in research and scientific education over the past five years. The contribution, actual and potential, of Chinese science is probably greater now than at any time since its eclipse in the seventeenth century by the mathematization of physics in Europe.

Coldest winter turns to spring

The ravages wrought on Chinese science between 1966 and 1976 by the demented anti-intellectualism of the Cultural Revolution as followed by the Gang of Four are now familiar. (For a comprehensive account of science in China, 1966–78, see ref. 1). Universities and basic research establishments were closed; intellectuals in general and scientists in particular were vilified and humiliated as class enemies inspired only by personal ambition; anything foreign was automatically tainted: Einstein was denounced as a bourgeois intellectual and relativity was ridiculed. Although a handful of prestigious or strategic research projects (notably in the defence sector) were protected, the great majority were either cancelled or directed, with disastrous results, by ideologically selected cadres with no scientific education or experience; activity in some fields at the height of the frenzy was limited to seminars and discussions organized secretly and at great risk by a dedicated few. Researchers often had to justify their work with putative practical applications so that, for example, an ornithological study published in 1973 caused outrage in the west by pointing to the culinary values of various threatened species. Genuine scientific writing virtually ceased for several years.

This disruption did immense damage. Perhaps most serious was the loss of scientific personnel. Almost all universities had closed by 1967 and although some courses

and applied research had resumed by 1973, the first batch of thoroughly trained graduates did not emerge until autumn 1981. It is the generation lost in this period which, now in its late twenties and early thirties, should be providing the creative thrust for Chinese research. (It is estimated, for example, that only 0.7 per cent of Chinese aged over 25 are university graduates, compared with some 5.5 per cent in Japan). To make matters worse, the preceding generation of scientists already productive by 1966 also sustained heavy losses. A large and unrecorded number were systematically broken in body and spirit by years of manual labour, bullying and humiliation often in remote and inhospitable regions. An official Chinese document has estimated that some 70 per cent of senior academics in 14 medical colleges and 11 institutes under the Ministry of Public Health were falsely charged and persecuted².

Apart from the consequent severe shortage of trained manpower, it would not have been surprising had such intense repression induced a state of paralysis in Chinese science. Indeed, by the time the Gang of Four was removed in 1976, very little original work was under way. However, with the subsequent return of the pragmatic Deng Xiaoping and his supporters, new optimism grew as the leadership publicly identified the development of science and technology as the most fundamental of the 'four modernizations', without which the other three (agriculture, industry and defence) would be impossible. This position was formally endorsed and amplified by the seminal National Science Congress convened in Beijing in 1978¹. Attended by scientists and scientific administrators from all disciplines, levels and areas of China and addressed by senior leaders including Deng Xiaoping and Fang Yi, the congress reaffirmed the value of science and scientists to the community. Under the slogan 'Springtime for the people, springtime for science', it reestablished the respectability of expertise as opposed to blind ideological purity, elevated scientists from class enemies to members of the working class, guaranteed that rational and expert analysis would in future inform scientific decision-making, reanimated the scientific bureaucracy which had been established in the 1950s and identified research priorities for the coming eight years.

Perhaps the most significant aspect of the congress was its implicit recognition



The Great Wall

that while the prevailing interpretation of the socialist ethic should continue to characterize the behaviour and attitudes of researchers, scientific value judgements should nevertheless be freed from ideological shackles. Relativity was no longer fair game for political analysis. The congress implicitly served notice of the Party's intention to adopt a supervisory role, allowing day to day decision-making in science to revert to experts, in an atmosphere of open debate. This process was symbolized in May 1981 with the replacement of the politician Vice Minister Fang Yi by distinguished structural chemist Lu Jiaxi as President of the Chinese Academy of Sciences.

The conference remains a prominent reference point for Chinese science policy today. However, important changes have since taken place. The optimism inspired by the new post-Revolution attitude of the authorities and media towards science and technology, compounded with relief at the passing of chaos, engendered overconfidence and euphoria not without similarities to British optimism in the sixties about Harold Wilson's 'white heat of technology'. This has now been tempered by experience: over-ambitious research priorities have been replaced by others more consistent with development needs and structural changes in the bureaucracy have sought to ensure that future science and technology planning more diligently serves short and medium term economic goals.

How China runs its science

Not surprisingly for a planned economy, the administration of Chinese science differs most from that of Western countries in the degree of centralization



Hefei, in Anhui Province, is China's "science city".

attempted. The system strongly resembles that of the Soviet Union in the 1950s on which it was modelled. Guidelines for science and technology are drawn up by the State Economic Commission, as part of China's overall development strategy. Policy is then formulated and implemented by the State Science and Technology Commission (SSTC), which is essentially a ministry of science and technology. Its primary functions include:

(1) Detailed formulation of national research and development priorities and allocation of funds accordingly. Proposals are drawn up on policy, development, personnel planning, import of technology and coordination of research and finance by more than 60 specialist panels of scientists and administrators, relying increasingly on peer assessments and reviews.

(2) Coordination, partly through the provincial science and technology commissions subordinate to it, of research and development projects to ensure that priorities are reflected and duplication minimized.

(3) Guidelines for science and technology are drawn up by the State Planning Commission, in consultation with the State Economic Commission.

Although it commissions some large-scale strategic projects, the SSTC itself does not directly control subordinate laboratories. The major agencies of research are:

(1) The Chinese Academy of Sciences (CAS or Academia Sinica). This is the traditional stronghold of Chinese basic research. Although it is technically subject to SSTC control, its great prestige and direct links with senior leaders probably allow it considerable freedom. CAS draws

up its own basic research priorities, which guide the selection of research projects for its 117 research institutes³ encompassing between them most traditional areas of the natural sciences. CAS has an annual budget of around £180 million.

(2) The universities. Since the establishment of the present system of higher education in the 1950s, again on Soviet lines, universities have focused largely on teaching rather than research. This is now changing rapidly. University basic research is beginning to compete in quantity and quality with that of CAS, and universities are also becoming prominent in applied fields, especially engineering. The number of universities, currently 704 is also increasing⁴.

(3) Central government. Many ministries and central organs have their own institutes and technical colleges, which form a large proportion of the applied research sector. Many government research and development subscribers control academies comprising specialist institutes — for example, there are some 20 research institutes in the Chinese Academy of Medical Sciences under the Ministry of Public Health. An important special case is defence research and development, under the National Defence Commission for Science, Technology and Industry (separate from and parallel to SSTC).

(4) Industry. Many factories and state enterprises maintain their own development laboratories, sometimes with government financial assistance.

(5) Regional science and technology commissions requirements of each province are identified and met by the provincial STCs, which commonly maintain around 150 research institutes, often in coordination with other local organs such

as provincial agricultural commissions. Projects are generally applied and closely dictated by the demands of local industry and agriculture. A similar more rudimentary structure operates at prefecture and county levels.

This largely structural description is limited by the difficulty of obtaining budget statistics, and of interpreting such data as are available in the light of China's socialist accounting methods. In 1978, the last year for which figures are available, total research and development expenditure was estimated at £5,300 million, representing less than 1 per cent of gross national product. It is likely that this proportion is now significantly higher.

Another increasingly important body in Chinese science is the China Association of Science and Technology (CAST). An umbrella organization for the growing number of specialist societies, with a total membership of over 1.1 million it has the following three main functions:

(1) Popularization of science. CAST seeks to raise the level of scientific awareness amongst the population, in order to increase receptiveness to new ideas and eliminate conservatism and reliance on traditional methods (especially prevalent in agriculture). To this end, it organizes public lectures, seminars and demonstrations and helps to orchestrate an intense media campaign to educate the public.

(2) Inter- and intradisciplinary communication. The compartmentalization of Chinese science together with the relative immobility of scientific personnel have engendered severe problems of communication between scientists in related fields. The societies under CAST seek to promote the flow of ideas by organizing specialist symposia and academic exchanges and publishing academic periodicals.

(3) Provision of science and technology advice and services to government. In 1978, societies under CAST began to undertake consultancy projects which are becoming increasingly popular. For example, the site for the Yellow Sea deepwater port now under construction at Shijiusuo was chosen on the advice of a feasibility study by the Chinese Society of Oceanography and Limnology⁵.

The "four modernizations"

The central policy objective at present is to ensure that science and technology "serve economic construction"⁶, meaning that although "research on basic science should not be neglected"⁷, the emphasis is strongly on applied work. This is reflected in current priority areas. It is now recognized that the programme formulated at the National Science Congress was beset by "the shortcomings of making too rash demands, setting too high targets and envisaging too large scales". Consequently, for example, plans to build a 50 GeV proton synchrotron by 1985 have been

shelved, as has an ambitious fusion energy project based on a tokamak device similar to the Joint European Torus, and sophisticated fields such as genetic engineering will for the time being receive less support than envisaged by the National Science Congress. But most other aspects of the 1978 programme¹ survive in the SSTC's recently announced priority areas within China's 15-year economic development plan due to begin in 1985⁷. These are:

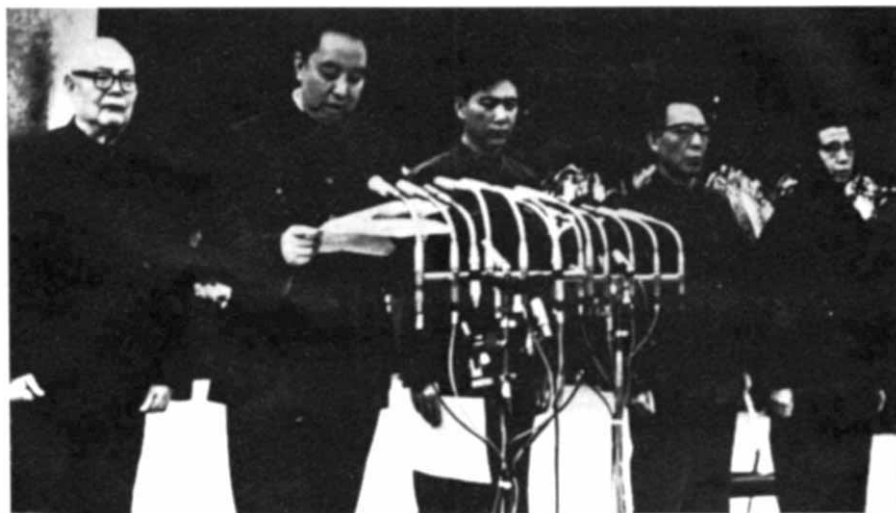
- Agriculture (plant breeding, forestry, ecology and husbandry)
- Energy (exploration and conservation, energy economics for large-scale consumers, coal, hydraulic power, off-shore development)
- Raw materials
- New technology (lasers, cryogenics, infrared spectroscopy, electronics and computers)
- Transport
- Communications
- Food
- Textiles
- Machinery
- Social sciences

In addition, considerable resources are being devoted to improving mechanisms for the wider application in industry and science of research achievements: this is the aim of the so-called "four transfers", from laboratory to production line, from military to civil, from advanced laboratories and enterprises to less advanced ones and from abroad to China. And at the planning level, the SSTC has recently lost part of its competence to the State Economic Commission (which is drawing up the development plans), in order to tie science and technology planning more closely to development aims.

Basic research

The shift away from basic research, which now accounts for around 5 per cent of total spending on civil scientific research, has not surprisingly generated opposition from researchers themselves, and from politicians who had hoped to enhance China's international prestige with high technology projects such as the 50 GeV synchrotron. Nevertheless, it is now widely accepted that a sensible balance has been struck: indeed the accelerator programme was initiated against the advice of many eminent Chinese and foreign particle physicists who felt that China would do better to use existing facilities overseas. Current policies are perhaps influenced by the obvious and, to some Chinese, embarrassing success of neighbouring Japan, in turning foreign basic research into domestic production capability. But Chinese planners have also noted that Japan is now herself beginning to attach a higher priority to creativity in fundamental science, and it is likely that given political stability and economic growth, basic research in China will gradually expand.

How are these policies reflected in the



The new era begins — Premier Hua Kuo-feng reads Chairman Mao's funeral oration. Flanking him (left to right) are Yeh Chien-ying, Wang Hung-wen, Chang Chun-chiao, and Mao's widow Chiang ching.

selection of research topics? Although scientists are invited to apply for funds to pursue their specific interests, the likelihood of economic benefits is always a major criterion in deciding whether to grant applications; furthermore, the majority of university and CAST research projects are formulated and handed down by the central scientific authorities although eminent researchers often participate directly in the preceding consultative process. Thus, for example, zoological and botanical taxonomy receive strong support because: (1) they form part of the vast categorization which is currently under way of Chinese natural resources; and (2) they require little expensive foreign equipment.

The taxonomic findings are then built upon with extensive investigations into chemical and medical uses of plant and animal products. Chinese zoology and botany are not advanced in Western terms, due to lack of modern equipment and modern techniques. However, many new uses have been found for plant and animal products, especially in medicine, and areas such as phytochemistry have been strengthened by the development of new purification and analytical techniques.

In astronomy, China's lack of equipment limits her international contribution largely to the work of a growing number of brilliant theoreticians, while the experimental emphasis is on areas such as astronomical timekeeping and calendar correction, observations of satellites and solar physics.

Some institutes established originally for fundamental research have hastily diverted their resources to applied projects, so that for example, institutes of palaeontology now vigorously apply palaeontological knowledge and dating techniques to mineral prospecting, and institutes covering biological sciences usually have departments devoted to studying the economic uses of their subject matter. The general impression is rather ungainly: there is sometimes little in common between pure

and applied projects within a given establishment. But this is largely due to the rapid and dramatic changes since 1966. A more rational research structure, better reflecting worldwide interests, will gradually emerge with experience, accelerated particularly by ever growing scientific contacts with the West.

China's scientific door swung open following the National Science Congress¹. Contacts with the West, and particularly with the United States, Japan, West Germany and France, have grown rapidly. They range from the placing of Chinese research students in Western universities to exchanges of visiting scholars and large-scale collaborative research projects such as the Sino-French geotectonic survey of Tibet, reported in this journal⁸. Such exchanges are seen by most Chinese as a quick, and cheap, way of catching up with the developments that China ignored during the Cultural Revolution, and of educating China's brightest young scientists. The programme is limited by China's shortage of foreign exchange, but this has been alleviated by the generosity of some foreign governments, universities and foundations. Moreover, the benefits do not flow all in one direction. In many fields — geophysics, epidemiology, ecology, botany and meteorology to name a random selection — China's unique experimental conditions provide a strong incentive to Western researchers. In others, as diverse as biochemistry, microsurgery and pig breeding, China already has groups and techniques that lead the world.

Strengths and weaknesses

It has been said that the great strength of Soviet science is its ability to orchestrate large scale interdisciplinary projects given political impetus at a sufficiently high level. This is certainly true in China. One of the most successful areas in Chinese fundamental science since 1949 has been the synthesis of biochemical molecules, notably bovine insulin and yeast alanine



Chuanshan University, Canton. China boasts 704 universities and the number is increasing. Most are controlled by the Ministry of Education.

t-RNA. Both of these achievements required long and painstaking collaboration between many establishments.

The tRNA synthesis, announced in January last year⁹, merits closer attention. Work began in 1968, but owing to the disruption of science at the time, little progress is said to have been made before 1976. The 76-nucleotide molecule was synthesized by a combination of chemical and enzymatic techniques, through collaboration between the Shanghai Institutes of Biochemistry, Cell Biology and Organic Chemistry, the Peking Institute of Biophysics (all under CAS), Peking University Biology Department (under the Ministry of Education) and the Shanghai No. 2 reagent factory. Few conceptual advances were made; the significance of the task lies rather in its sheer complexity and the extent of collaboration between different groups¹⁰. The ultimate aim of the work is said to be gene synthesis by chemical methods; funding is justified in terms of medical applications.

This organizational strength of the Chinese system has also been demonstrated in other fields. China is good at large-scale surveys and has, for example, recently published important maps showing the distribution of types of cancer and other diseases. Intensive geographical surveys are regularly carried out in collaboration between diverse groups to map geological, pedological, climatological, hydrological and other properties of large regions.

It is ironic that the vertically oriented centralized structure that gives Chinese science this organizational strength is also largely responsible for its major weaknesses — inflexibility and compartmentalization, also part of the Soviet legacy. For without high level support in pursuit of a specific objective, communication between related disciplines, and even within single disciplines, is poor.

In fundamental science, this problem is illustrated by the gulf between CAS institutes and the universities, many of

whose research interests now overlap considerably. The problem is partly due to the employment system, whereby researchers spend lifetimes at single establishments, with little opportunity for cross-fertilization. Furthermore, now that the universities are once again producing graduates, the gulf threatens to widen. As in other socialist economies, there is no job market: jobs for graduates are rather allocated according to Ministry of Education quotas by the universities, which can cream off their brightest students for their own laboratories. So although basic research in CAS institutes is generally better funded and equipped than that in universities, it often has to make do without China's brightest young graduates.

Another obstacle to cross-fertilization throughout Chinese science is the degree of specialization of most research establishments, so that members of the largely static staffs have little opportunity to meet workers in other fields (or even those in the same field but working at different laboratories), thus hindering the development of interdisciplinary subjects such as bio-engineering and environmental sciences. And within universities, teaching and research are usually undertaken largely by different groups of staff, so that graduates often have little appreciation for the demands of research work and researchers sometimes lose touch with the fundamental intellectual structures underlying their own fields.

Perhaps the most damaging effect of compartmentalization is the widespread wasteful duplication of effort. This is particularly prevalent in general areas which have been vaguely identified as priorities without closer definition and coordination of specific objectives: good examples are lasers and the applications of remote sensing, which are studied ubiquitously and with great vigour but with inadequate coordination under CAS, universities and some ministries. An important special case

is the wide gulf between civil and military research: because of its completely separate administration, compounded by the secrecy with which it is conducted, the latter has gradually expanded independently into areas of purely civil interest, often unbeknownst to civil researchers.

Most of these problems are by no means unique to China; more important, most have now been identified as deficiencies and steps are being taken to overcome them. For example, CAS is now strongly advocating greater mobility for the staff of its institutes, university teachers are being encouraged to do more research (and vice versa) and organizations such as CAST seek to promote the "four transfers" referred to above.

However, there is another class of more insidious problems with their roots rather in the ideological contortions from which China has recently emerged than in the structural elements of the Soviet legacy. China's expanding scientific contacts with the West provide an example. Although the door remains firmly open, the light which shines through illuminates a crucial dilemma. The management of contacts with the outside world has for centuries been one of the major fault lines of Chinese society. The official position remains that Western culture, habits and lifestyles constitute a decadent and poisonous influence which must be shunned at all costs. This position will become more difficult to maintain as personal contacts and friendships form between Chinese intellectuals and foreign colleagues, encouraged by official recognition of the need for scientific exchanges with the West.

A second fault line divides researchers themselves and some of the cadres who control the administration of science and technology, many of whom rose to responsible positions during the Cultural Revolution, are identified with the policies of that time and have little understanding of science. The friction between them, generated in the Cultural Revolution, will take a long time to dissipate.

Legacy of revolution

Another problem with origins in the Cultural Revolution concerns the quality and deployment of scientific personnel. During the turmoil, many qualified researchers were transferred to applied work in which they had no experience or expertise; the task of identifying and relocating them has not been completed, and we have already seen that new graduates are not always optimally placed. Meanwhile, many posts in scientific institutions continue to be held by unproductive and untrained staff appointed for political reasons during the Cultural Revolution, accounting for the apparent average staff: student ratio of 1:3 at Chinese universities. Furthermore, trained researchers do not yet have the same incentives for creativity



A light-pen graphic alphanumeric display produced by a radio factory in Hunan Province.

as Western colleagues, either materially or in exposure to peer acclaim. Scientists are still encouraged modestly to avoid the limelight: heroes are made of those who try to avoid taking credit for their work. However, the SSTC has now sensibly resumed the award of scientific prizes (instituted before the Cultural Revolution) for outstanding research. In addition, there are now those who advocate a certain amount of competition between laboratories, despite the risks of duplicating resources and discouraging the dissemination of preliminary results.

Underlying all of these issues, perhaps the most important sociological question remains the status of the scientist and intellectual in Chinese society. Although no longer seen as class enemies, many scientists are still paid perhaps 20 per cent less than many factory workers, who, furthermore, can now often double their incomes with bonus earnings. The current media campaign emphasizing the need for better material conditions for intellectuals probably reflects a deep-seated concern that this disparity may direct new talent away from science.

Prognosis

These problems, however daunting, should not be exaggerated. They are perhaps less important than the sheer geographical and material obstacles to Chinese science: communications in many areas are poor and even the most prestigious laboratories cannot afford the sophisticated equipment of their western counterparts: there is a particular dearth of computing facilities and expertise. Nevertheless, the optimism and enthusiasm which prevail in most areas of Chinese research appear to be justified by the startling progress of the past five years. Most of the problems outlined above have been identified, widely debated and are beginning to be tackled with flexible and often controversial measures. The key impor-

tance of rapid improvement and expansion in scientific higher education has been recognized: visitors to Chinese universities who are aware of the disruption of less than a decade ago are impressed by the quality both of teaching and research, the atmosphere of enthusiasm and industry and often by what has so far been achieved with rudimentary experimental resources. Comparisons with other developing countries such as India, where a few centres of excellence tower over a minimally evolved scientific infrastructure, are usually favourable.

China's new pragmatic approach to science is exemplified by the growth of the basic research sector around the rather obscure city of Hefei, capital of Anhui Province. A small village before 1949, Hefei is a new town with a population of around 0.5 million. However, despite a relatively prosperous agricultural hinterland and recent growth in local light industry, its industrial base is weak and communications, especially with China's developed eastern seaboard, are not well developed. Therefore, when the decision was taken in the Cultural Revolution to decentralize science, rendering it (as it was argued) less vulnerable to Soviet attack and at the same time bringing it closer to the masses, Hefei was chosen as a suitable site for the relocation of the University of Science and Technology of China (USTC) formerly based in Beijing. Other important institutes were also moved or newly established there, including the fusion project (now the CAS Institute of Plasma Physics). By the time that the drawbacks of these policies were realized, Hefei's scientific identity was already established and it would now be impracticable to return the Hefei establishments to their original homes.

Instead, the opposite course is being taken. Basic science in Hefei is flourishing. The research institutes, which occupy a large self-contained site some 20 miles from

town, are being expanded and reequipped. Although the original fusion project has been scrapped, the Institute of Plasma Physics is still constructing a smaller torus, with the benefit of impressive engineering workshops. A new Institute of Solid State Physics is under construction, and the fledgling Institute of Intelligent Machines will shortly move there from the centre of town. The aim is to construct a "scientific base area" which will become a self-contained centre of excellence, better equipped, and drawing on better talent, than most Chinese research establishments.

Elitism allowed

But perhaps the most exciting institution in Hefei is the USTC. The only university remaining under the direct control of CAS, better equipped and more imaginatively run by younger staff than most of its counterparts, it enjoys increasing success as a cradle for China's scientific elite. Of some 100 young physics graduates from all over China selected in 1982 by Professor T.D. Li for further study in the United States, 22 were from USTC, including those who came first, second, third and fifth in the selection process; last year 66 per cent of USTC's graduates passed the national examination for postgraduate positions compared with a national average of well under 20 per cent. Such impressive statistics have not been achieved without controversy. USTC's relative unorthodoxy is resented in some circles; attempts were recently made to close its juvenile class, which draws on prodigies as young as 11 years of age from Chinese schools for early university entrance. The class is still open, but has no counterpart in other Chinese universities. But whatever the ideological anomalies of USTC, it is rapidly becoming one of China's most important educational institutions.

By Western standards, Chinese science, taken as a whole, remains relatively backward. It is impossible to predict when, and under what circumstances, China will be able to claim that the "four modernizations" have been achieved. But when they are, it is certain that basic science will have played a large part, and that the role of the scientist will be recognized. In the Cultural Revolution, there were many proverbs in wide use advising against excellence or prominence in any guise. It was said for instance that tall trees bore the brunt of strong winds. The creative individual in science is now rewarded (albeit modestly) rather than vilified, and at Hefei, tall trees are again being planted. □

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A giant leap for physics — where to?

Physicists in Geneva have discovered the intermediate vector boson; and in an experiment under Lake Eyrie the proton has failed to decay.

ONE of the gods of high-energy physics has revealed himself: the intermediate vector boson, the massive counterpart (for the weak force) of the photon (for the electromagnetic force), a particle predicted by the Salam–Weinberg unified theory of the two forces. But despite the Nobel Prize that will no doubt follow for at least one of the 180 experimenters involved, the two groups that discovered the particle at the European Centre for Nuclear Research (CERN) in Geneva were still arguing last week about whether to admit they had found it. The official phrase is that the data “begins to reveal the expected signature” of the particle.

The problem is a very commendable degree of scientific professionalism, which may yet prove wise: according to Alan Astbury, the cautious British spokesman for one of the groups, the intermediate vector boson is the most desired and well predicted object in the history of particle physics. It certainly falls in the class of the pion, predicted by Yukawa, and the Ω^- predicted by Gell-Mann. And its discovery would be a crucial sign of the virility of contemporary theoretical physics. It is thus all too easy to jump to conclusions. Wary physicists remember the muon — a particle loudly hailed as the pion until it turned out to be the new and totally unpredicted heavy electron.

But once that is put neatly on the record like an insurance premium against disaster, the result is almost certainly a triumph. It is a triumph in particular for Carlo Rubbia, the Italian-American whose irascible dynamism and self-confidence drove CERN into building the facility that eventually created the particle, the proton–antiproton collider. It’s a triumph for CERN management, it’s a triumph for the physicists and engineers who designed and built the collider (in particular one extraordinary and crucial part of it, which grasps a hot ‘gas’ of antiprotons and cools it before acceleration). It’s a triumph for Europe, too, for this discovery comes well ahead of any American, Soviet or Japanese competition.

So what has been discovered? Experimentally, nine events among thousands of millions of others have shown the collision at 540 GeV (in the centre of mass) of a proton and antiproton. As usual, these 1-GeV-mass particles ‘fragment’, each creating a shower of particles. One shower follows the line of the antiproton, the other the proton. But in the nine events there is

something else: the track, in some cases of an electron and in others of a positron — the antiparticle of the electron — shoots off nearly at right angles to the other tracks and at high energy (20–40 GeV). Nothing balances it on the other side.

This is a textbook example of what Rubbia wanted to see. Even Astbury admits “you could teach a schoolchild with these”. The interpretation: one of the three quarks in the proton has collided ‘head-on’ with one of the three antiquarks in the antiproton. Interacting by the weak force, the particles have created an 80-GeV-mass charged W, one of the intermediate vector bosons. Because the quarks have only a fraction of the total proton kinetic energy, just enough to create a W, it is almost at rest in the laboratory. The W has then decayed into an electron (or positron) and a neutrino, which have shared the mass-energy of the W and gone in opposite directions. The neutrino went undetected, as neutrinos interact very rarely with matter. The electron (or positron) was detected whenever it was out of line with the fragmentation showers of the rest of the proton and antiproton.

The mass of the W from these few events is calculated to be 81 ± 5 GeV, bang on target for the 82 ± 2 GeV prediction of the Salam–Weinberg model. The production rate of the particle is also just as predicted. The neutral partner of the W, the Z, which should weigh 92 ± 2 GeV according to Salam–Weinberg, is produced at a ten times lower rate, and should be seen in the next collider run at CERN, which will take place about Easter and should bring in ten times more events. Easter is also the time when the more precise theoretical predictions for the intermediate vector bosons will be tested, and when we shall see whether caution was justified: for example, it will be determined whether the particle decays violate left–right symmetry (that is, break ‘parity’) as expected from the weak interaction.

Meanwhile, another important result for particle physics was announced last week: a lower limit on the proton lifetime determined by the Irvine–Michigan–Brookhaven collaboration down a salt mine 2,000 ft beneath Lake Eyrie. This places the lifetime of proton against positron– π^0 decay at more than 6.5×10^{31} years. This is nearly an order of magnitude longer than the longest prediction of minimal SU(5), one of the simplest of the ‘grand unified theories’, or GUTs, which

links all the non-gravitational forces in a unified scheme, and so throws that model into doubt. “Supersymmetric” theories, which are more favoured among theorists these days, and also bear on gravity, are as yet untouched by the result.

The result is significant in being the first to begin to sort out the wheat from the chaff among the many different GUTs — and the experiment promises more, stretching the detection limit to a lifetime of 10^{33} years, and seeking other decay modes (including the kaonic modes predicted by supersymmetry) in the next three years of running. ‘Detections’ of proton decay in other competing experiments (under Mont Blanc and in an Indian gold mine) may be neutrino events from cosmic rays, the group believes.

So a marvellous week for particle physics — but what does it all mean? Clearly the subject — which underlies all physics — is at one of the most creative phases in its history. On the whole, at accelerator energies, theory is leading the subject, while experiment appears merely to confirm. But way up ahead, at the 10^{15} GeV energy regions probed by the GUTs, where only proton and neutron decay experiments can shed observational light, there are worms in the golden apple. There is not one GUT to test, like a theory of general relativity, but several. The principal reason is that an essential sector of the theories is being neglected: the Higgs fields, which fill space-time and gnaw away at an underlying symmetry of forces to break it into the very different forces we observe. The symmetry may be beautiful; and the Higgs fields may exist (they are needed in the Salam–Weinberg model, which now appears to be confirmed). But what are the Higgs fields? All the untidy problems — such as the matter of why the intermediate vector bosons are heavy — are essentially disposed of as properties of the Higgs fields.

Physics now appears like a tapestry: beautiful on the surface, but full of knots and crossed threads behind. It will remain so until the problem of the Higgs fields is solved.

Robert Walgate

On page 287, Frank Close describes the historical background to the concept of the intermediate vector boson. Dr Close wrote just before last week’s news broke.

Optoacoustic detection

Revival of an old technique

from A. I. Ferguson

THE optoacoustic or photoacoustic effect, an intriguing example of which is described¹ on p.321 of this issue of *Nature*, is proving an important and sensitive technique for studying the interaction of light with matter. The effect was discovered over one hundred years ago by Alexander Graham Bell² but, after a period of rather intense study when an attempt was made to develop a 'photophone' (see inset), the effect was dismissed as a curiosity. In the past decade there has been a revival of interest in optoacoustic phenomena, and now, with the availability of lasers and the development of sensitive microphones, the optoacoustic effect can be used as a valuable probe in such diverse scientific fields as physics, chemistry, biology and medicine^{3,4}. There is also a growing interest in applying the techniques in the food and drug industries and in agricultural science.

In Bell's original observation, sunlight was focused on to a selenium photocell. Although Bell was primarily interested in detecting the electrical changes in the selenium cell as the sunlight was chopped, he found that sound at this chopping frequency could be heard by a nearby observer. Modern optoacoustic experiments bear a striking resemblance to this. The modern light source is more likely to be an intense arc lamp or laser and the acoustic signals are detected by having an exchange gas which couples the sample and the microphone. Heating and cooling of the sample heats and cools the gas and the resulting changes in pressure are detected in the microphone. In his book *Photoacoustics and Photoacoustic Spectroscopy*⁴, Rosencwaig reviews many applications of this method in samples ranging from single crystals through powders of inorganic insulators to biological tissue. A useful feature of the gas microphone technique is that by varying the chopping frequency of the light it is possible to obtain depth profile information about the sample.

In the experiments described in this issue of *Nature*¹, O'Connor and Diebold have detected absorption in $\text{Cl}_2/\text{H}_2/\text{N}_2$ mixtures using a gas microphone. By shining an argon ion laser at 476 nm into the gas mixture they find an enhancement — of about 10^3 — of the optoacoustic signal over that obtained when H_2 is absent. The enhancement has been attributed to the photodissociation of Cl_2 followed by a chain reaction involving H_2 and Cl . Thus a small amount of laser energy releases a large amount of chemical energy. Indeed, the authors report that explosions take place when the laser power exceeds about 10 mW — this must be the ultimate in opto-

acoustic detection!

By monitoring the optoacoustic signal as a function of time it is possible to observe the concentration of the reactants as the reaction takes place. A tantalizing possibility is that the speed of the chain reaction could be monitored by observing the optoacoustic signal as a function of chopping frequency. Although the authors suggest such investigations they have not yet carried them out.

Optoacoustic detection is by no means limited to the use of conventional microphones. Indeed, some of the smallest detected absorptions have been measured using other methods — for example, detection of direct strain measurements and detection of refractive index gradients.

In the direct strain method, a piezoceramic is bonded to the sample. Strains induced in the sample by the absorbed radiation can be directly measured as a voltage across the piezoceramic. Absorptions of as small as 1 part in 10^7 have been detected using this technique when the sample is illuminated with a pulsed dye laser⁵.

Träger *et al.*⁶ have recently shown that the method can be used to study surface interactions. Using a silver-coated piezoceramic transducer they studied the absorption spectrum of SF_6 laid down on the silver surface. They have found that they can detect surface adsorption of SF_6 down to 0.1 of a monolayer. These studies could have important implications for the semiconductor fabrication industry.

Techniques based on the detection of a refractive index gradient have the

advantage that they involve no direct contact and so may be used on very hostile samples, for example, plasmas or flames. In this case the local heating and cooling causes local changes in the refractive index of the sample. A second light source, usually a low-power laser, then probes the excitation region. The gradient of the refractive index causes changes in the direction of the probe beam which can be easily detected. A recent publication by Zapka *et al.*⁷ has demonstrated the use of this method in spatially resolving and measuring the temperature of a flame by measuring the arrival time of an acoustic pulse. The technique could be applied to observing other forms of combustion.

The range of potential applications of optoacoustic detection is enormous. Many of the examples of optoacoustic phenomena described in the literature have simply been demonstrations. As the method matures and is more widely appreciated it will inevitably become an indispensable tool for the laboratory scientist in disciplines ranging from the esoteric to the highly practical. Many of these new applications will be explored at the 'Third International Topical Meeting on Photoacoustic Spectroscopy' which is to be held in Aussois, France in May. □

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BELL'S PHOTOPHONE

OUR readers are already aware that the object of the photophone is the transmission of sounds both musical and vocal to a distance by the agency of a beam of light of varying intensity . . . not the least of the remarkable points in this research is the discovery that audible vibrations are set up in thin disks of almost every kind of material by merely throwing upon them an intermittent light. Hence in theory, if not in practice, the receiver may be reduced to the divine simplicity of a mere disk of vulcanite or of zinc, on one side of which the vibrating beam of light falls, and at the other side of which the hearer listens.

We may remind our readers here that this apparent direct conversion of light into sound takes place, as Prof. Bell found, in disks of all kinds of substances — hard rubber, zinc, antimony, selenium, ivory, parchment, wood, and that he has lately found that disks of carbon and of thin

glass, which he formerly thought exceptions to this property, do also behave in the same way.

We may perhaps remark without impropriety that it is extremely improbable that the apparent conversion of light into sound is by any means a direct process. It is well known that luminiferous rays, when absorbed at the surface of a medium, warm that surface slightly, and must therefore produce physical and molecular actions in its structure. If it can be shown that this warming effect and an intermediate cooling by conduction can go on with such excessive rapidity that beams of light falling on the surface at intervals less than the hundredth of a second apart produce a discontinuous molecular action of alternate expansion and contraction, then the mysterious property of matter revealed by these experiments is accounted for.

As reported in *Nature*, 4 November 1880.

The search for bosons

A golden year for the weak force

from Frank Close

IT is common at this time of year to make predictions for the next twelve months and hope that readers' memories are short. Particle physicists confidently expect that a singularly important discovery concerning the nature of the weak force will occur in 1983. To prepare readers for this possible event, here is a preliminary briefing about the history of this force and the reason for the current excitement.

The weak force, responsible for the β -decay of neutrons to a proton, an electron and an antineutrino and for the interactions of neutrinos, was an enigma until some dramatic advances were made in the late 1960s and early 1970s. A successful theory uniting the weak force with the electromagnetic forces was constructed by Sheldon Glashow, Abdus Salam and Steven Weinberg for which they won the 1979 Nobel Prize for Physics. Just as electromagnetic forces are transmitted by photons, so, according to their theory, the weak forces are transmitted by W^+ , W^- and Z^0 bosons. Whereas photons are massless and freely emitted, the W and Z bosons are predicted to have huge masses, some 80–90 times greater than that of neutrons or protons, and it has not so far been possible to produce them in the laboratory. Experiments in progress at CERN, Geneva are expected to achieve the required conditions for their production, however, and their existence (and the electroweak unification theory) could be experimentally confirmed by the summer.

In 1933, quarks — the constituents of protons and neutrons — were unknown; the neutron and proton were believed to be structureless elementary particles and Fermi produced the first theory of the weak force in an attempt to understand neutron β -decay (*Ricerca Scient.* 4 (2), 491; 1933). His idea was inspired by quantum electrodynamics where a neutron or proton absorbs a photon at a single point in space-time and preserves its electrical charge. Fermi proposed that an analogous process of charge conservation occurred in β -decay: the change of charge in the decay of the neutron into a proton is caused by the emission of an electron and an antineutrino at a point. The electron and antineutrino each have spin $\frac{1}{2}$ (measured in quantum units) and so their combination can have spin 0 or 1, depending on their orientations. The photon, by contrast, has spin 1. By analogy with electromagnetism Fermi had (correctly) supposed that only the spin 1 combination emerged in the weak decay. To further the analogy, beyond the need for charge and spin conservation, O. Klein suggested, in 1938, that a spin 1 particle (' W boson') mediated the decay — the boson having a role in

weak interactions like that of the photon in electromagnetism.

In 1957 Julian Schwinger extended these ideas and attempted to build a unified model of electromagnetic and weak interactions by analogy with Yukawa's model of the strong nuclear force that binds atomic nuclei. As the pions π^+ , π^- and π^0 are exchanged between interacting particles in Yukawa's model of the nuclear force, so might the W^+ , W^- and γ (photons) be in the weak and electromagnetic forces. However, the analogy is not perfect in that the strong nuclear forces are charge independent whereas the weak and electromagnetic forces manifestly are not: the weak processes mediated by W^+ or W^- are much weaker than the γ -mediated electromagnetic interactions.

Schwinger realized that this difference in strengths is subtle and to some extent illusory. When we think of the weak force as feeble, it is because we are describing it in the Fermi way where the interaction occurs at a single point in space and time. In the Klein and Schwinger picture the interaction occurs between distant particles much as electromagnetic interactions do, the photon messenger being replaced by a W . The apparent strength of the weak force then depends not only on the strength of the W interaction with matter but also on the mass of the W ; the more massive the W the more feeble would the interaction appear to be. If the W couples to matter with electromagnetic strength then its mass must be of the order of 10–100 GeV, if the neutron half life and other observed weak interaction effects, such as the interaction of neutrinos, are to be understood. The essential mechanism underlying all of this is the uncertainty principle, the details of which are not necessary here.

However, there were still flaws in the idea. W^+ and W^- both interact with neutrinos, and photons do not: a further neutral boson was required (' Z^0 ') which can couple to neutrinos as well as other matter. This idea was first developed by Glashow in 1964 and then extended by Weinberg and Salam. They showed that the masses of Z^0 and W^+ , W^- could be mutually related, in particular the Z^0 cannot be lighter than W^+ and W^- (and so cannot be the photon).

Brilliant mathematical work by Gerard 'tHooft in 1971 proved that this theory of weak and electromagnetic forces was self-consistent. Experimental confirmation soon followed with the discoveries of processes mediated by the Z^0 , in particular the elastic scattering of neutrinos ('weak neutral currents'), and of parity violation in atomic and electron scattering. A decade

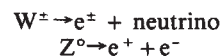
of successes culminated in the 1979 Nobel Prize for its creators — Glashow, Salam and Weinberg. However its most dramatic consequence — that W^+ , W^- and Z^0 bosons must exist — awaits direct confirmation.

Precision studies of processes mediated by W^+ , W^- or Z^0 suggested that the Z^0 should be about 10 per cent heavier than the equally massive W^+ and W^- . If the weak and electromagnetic interactions are unified (in the sense that they have identical intrinsic strengths), then

$$M_{W^+} = M_{W^-} = 82 \pm 2 \text{ GeV} \\ M_{Z^0} = 92 \pm 2 \text{ GeV}$$

These are much too massive to have been produced in any man-made accelerator and so their non-appearance was quite in accord with the theory. However, 1983 will see a pivotal change. A machine at CERN was commissioned last year which should be capable of producing such particles. Experiments searching for them are in progress.

What is being looked for, and how long must we wait for news? At the super proton synchrotron (SPS) at Geneva protons and antiprotons can be collided head on and mutually annihilated. The incident beams have energies of up to 270 GeV each and so there is the possibility of extremely massive particles, such as Z^0 or $W^\pm$, being produced among the debris. The debris consists almost totally of pions produced by strong interactions; events containing W or Z are expected to be rather infrequent. Their identification is made possible by exploiting the fact that the W and Z are not stable and can decay, producing electrons and/or positrons:



So at CERN, physicists from Europe and the US are looking for examples of proton-antiproton annihilations where electrons or positrons are produced. The cleanest signal would be an event containing both an electron and a positron whose combined momenta and energies reveal them to be the decay products of a massive particle, such as the Z^0 . An accumulation of events where an electron and positron are present with combined energy-momentum characteristic of a 92-GeV source would indeed be dramatic, but is unlikely to occur for some considerable time. If the CERN machine operates with 100 per cent efficiency and the theorists predictions for the Z^0 production rate are correct, then a handful of such events might emerge by the summer.

The production of electron/positron and neutrino arising from $W^\pm$ production and decay is expected to be an order of magnitude more copious. Up to a dozen such events might already have occurred but identifying them as $W^\pm$ decay products is not easy. A particular difficulty is that the electrically neutral neutrino cannot be

detected, so one cannot reconstruct a $W^\pm$ mass directly; more work is needed.

The proton and antiproton collide head on in the laboratory so a massive W will be produced, nearly at rest in the laboratory. When it decays to an electron and a neutrino, the electron and neutrino will share the rest energy (82 GeV?) of the W . If this was the whole story then a plethora of 41-GeV electrons would be a clear signal. The W is, however, usually produced with some motion in the laboratory, albeit small, and so the electrons' momenta will be distributed around the 40-GeV region. Unfortunately, single energetic electrons can also be produced in the decays of fast-moving pions, kaons, charmed and other massive particles and can contaminate the signal considerably. A distribution of electron momenta must thus be obtained and

an enhancement sought for around 41 GeV — the signal for an 82-GeV W boson.

If nature does behave as the theorists believe and if there are no unforeseen experimental difficulties, then the W and Z should be established this year. If they are, it will be a fitting fiftieth anniversary of Fermi's first attempts to understand the weak interaction. So confident are theorists that the W and Z are there to be found that failure to detect them could be no less interesting than their discovery. Either way 1983 seems destined to be a golden jubilee for our understanding of the weak force and its hypothesized union with electromagnetism. □

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Egg incubation

The reproductive biology of mound-building birds

from Jared Diamond

AMONG all living birds there is only one family that neither incubates its eggs by body heat nor cares for the young after they are hatched. This family is the Megapodiidae, the remarkable mound-builders (megapodes or brush turkeys), of Australia, New Guinea and other Pacific islands that incubate their eggs by heat from one of three external sources: volcanoes, the Sun, or rotting vegetation.

The heated incubation mounds that give the family its English name are the largest nests built by any bird: up to 5 metres high and 18 metres long. The young fend for themselves immediately on hatching and never receive any parental care. When accounts of these habits reached Europe from the early sixteenth century onwards, the stories were dismissed as false, fabulous and fantastic by biologists willing to accept accounts of mermaids and sea monsters. Observations and experiments during the past few decades have provided more details of mound-builders' breeding habits, but raise as many questions as they answer¹⁻¹². Recent papers add mound-sharing and mound-parasitism to the list of fabulous and fantastic habits^{13,14}.

To understand why mound-builders use exogenous heat sources for incubation, one can begin with the opposite question. Why does a tropical bird need any heat source for incubation, other than the warm air itself? The reason is that the air temperature fluctuates daily between extremes that are either too hot or too cold for incubation. For example, at mounds of *Megapodius freycinet* on the Indonesian island of Komodo, the air temperature at night drops to 14°C, while during the day surface temperature rises to 45°C (ref. 12). These fluctuations in air and surface

temperature are, however, quickly evened out underground and become negligible before a depth of 1 metre is reached⁹. This principle is exploited almost exclusively by the mound-builders *Macrocephalon maleo* of Celebes, *Eulipoa wallacei* of the Moluccas and some individuals of the wide-ranging *M. freycinet*. These birds simply dig a hole in the ground, deposit an egg and cover it up again. The holes are usually made in sand beaches fully exposed to the Sun. *Macrocephalon* prefers black sand to white sand, presumably because black absorbs more sunlight and is warmer.

Volcanic heat is used as an alternative by the entire population of *Megapodius pritchardii* on Niuafu'ou Island of Tonga, and by many individuals of *M. freycinet* on Simbo⁴ and Savo Islands of the Solomons, at Paraso Hot Springs on Vella Lavella Island of the Solomons and on the Talasea Peninsula of New Britain. These megapodes select an area of the volcano where the soil temperature is around 37°C, excavate a burrow 1 or 2 metres deep, lay an egg and refill the burrow. A few individuals of *M. maleo* similarly use hot springs in the interior of Celebes³.

For megapodes without access to volcanoes or sunny beaches, the remaining solution is to use the heat released by rotting vegetation, exactly as in a gardener's compost heap. The simplest way of using this heat source is seen in the Solomon Islands, where some individuals of *M. freycinet* deposit their eggs in disintegrated rotting trunks of certain tree species. Much larger and more elaborate compost heaps are constructed by the genera *Talegalla* and *Aepyodius* in the cool mountains of New Guinea¹, by *Alectura* and *Leipoa* in Australia and by

Megapodius lapérouse of Palau and the Marianas and many individuals of *M. freycinet*. These birds rake up huge mounds of rotting leaves, in which the temperature can rise as high as 60°C.

Another example is *Leipoa ocellata*⁹ which lives in the arid interior of Australia, where the temperature fluctuates by 50°C seasonally and by up to 16°C during a single day. The birds build a mound and use several techniques to keep the mound temperature at 33 ± 1°C. In the winter, the male and female *Leipoa* dig a large hole about 3 metres in diameter and 1 metre deep and fill it with dead leaves and twigs. With the onset of the rains, fermentation begins and the temperature rises to 50–60°C. Each day the male digs the mound open in the cool early morning to allow the heat to escape. By early summer, the rate of fermentation is slower, the air temperature is warmer and the mound needs to be opened only every 2 or 3 days. In midsummer the soil surface temperature reaches 60°C, and the problem is now that of how to cool rather than to heat the mound. This is achieved by covering the mound with sand to insulate it from the Sun. Finally, when fermentation is exhausted by late summer, the male opens the mound at mid-day to let the Sun warm the interior and to shovel Sun-warmed sand into the mound. All the work is done with the birds' large feet, which give rise to the family's Latin name of Megapodiidae. Crome and Brown¹³ watched a 1-kg megapode dig up and move a 7-kg boulder. Frith⁹ found that it took him half an hour with a shovel to dig a hole equivalent to one that a male *Leipoa* had dug in one and a half hours with its feet.

That *Leipoa* actively regulates its mound temperature was proved by Frith's experiments⁹. Next to a mound that a *Leipoa* was successfully maintaining at 33°C, Frith dug two similar-sized holes, one of which he filled with sand, the other with compost. The sand-filled hole did not reach 32°C until late in the breeding season, while the compost-filled hole overheated to 41°C. Each night, when Frith went home, the male bird attempted to correct and improve Frith's badly designed compost-filled hole. Frith then buried in the bird's own mound an electric heater powered by a remote generator, so that the mound could be quickly heated by 15°C. When this experiment was performed in the spring, the male coped successfully by opening up the mound to let the heat out. When the experiment was performed in the summer, however, the bird made the mistake of covering the mound with more sand as if to insulate it. Evidently *Leipoa* responded to the heater on the assumption that excess heat in the spring means too much fermentation but that excess heat in the summer means too much Sun and too little insulation. For a bird ignorant of electric heaters, his mistake in the summer was at least an intelligent mistake.

The experiments demonstrate that

Leipoa possesses a 'thermometer' and thermoregulatory system accurate to 1°C. For *Leipoa* the thermometer is probably in the bill or tongue, as one can watch the male probing the mound with his bill before adjusting the mound. In *Alectura*, *Talegalla* and *Aepyodius*, however, the head is unfeathered and the naked skin itself probably serves as a thermometer, as *Alectura* probes by pushing the head and neck into the mound. When the female approaches and is ready to lay an egg, the male digs one or more holes in the mound which the female tests. Eventually the female is satisfied with a hole's temperature, lays an egg in the hole and covers it. The hole must be of the correct depth to permit hatching but avoid parboiling: the surface temperature of an *Aepyodius* mound is 15–19°C, the temperature deep in the mound is 40–60°C and eggs are laid at a depth where the temperature is 33–34°C.

Females lay 5–33 eggs in one breeding season, at intervals of several days to up to a week or more. Each egg hatches after 40–96 days. Eggs in a clutch do not hatch nearly simultaneously, as with other birds. Instead, the mound is an assembly line containing eggs at very different stages of readiness for hatching.

Megapode eggs and chicks are unusual in many respects. Compared with eggs of similar-sized 'normal' birds, megapode eggs are very large (weight 3.5 times normal), thin-shelled (half the normal thickness) and unusually permeable to gases¹⁵. The incubation period is 60 per cent longer than for normal birds. The eggs have the highest yolk content (67 per cent) of any birds' eggs.

The chick hatches at an advanced stage, fully feathered, able immediately to regulate its body temperature (unlike chicks of other birds) and able to run within 1 h, to flutter within 2 h and to fly strongly within 1 day of hatching. Chicks of other species hatch by cracking their shell with the egg tooth; megapode chicks hatch by kicking their shell open. The hatched megapode chick finds itself buried under up to 60 cm of soil and spends up to 15 h digging its way to the surface. Once it emerges into the daylight, it flutters off, finds its own food and is never cared for by its parents. The effort that the parents devote to the eggs contrasts with their complete disregard for the chicks. Frith observed how a male *Leipoa*, digging an egg-laying hole in its mound for its mate, happened to uncover a recently hatched chick (his offspring) at a depth of 30 cm and kicked it out like a piece of debris. The chick staggered off past its mother, who ignored it and proceeded to lay her egg⁹.

As one watches megapodes work month after month on their enormous mounds, one assumes that the effort must result in egg-hatching success far beyond that attained by other birds. In fact, success is no greater than in conventional birds that just sit on their eggs. The megapode egg is

exposed to a whole set of dangers. Some eggs fail to hatch because they are at the wrong temperature because of bad weather or faulty mound construction. Mounds are regularly mined for eggs by predators, notably foxes, humans and varanid lizards (the group that includes the Komodo dragon). Lincoln watched a Komodo dragon 2.7 m long digging into a mound while an adult megapode kicked soil back into the hole as fast as the lizard dug¹². On Savo Island the communal nesting beach used by megapodes is staked off into sections, each of which is the property of one man to mine. The Celebes government rents out egg-hunting space on *Macrocephalon* nesting beaches. Of chicks that survive weather and predators to hatch, some suffocate while digging towards the surface, especially in hot weather. Finally, night herons (*Nycticorax caledonicus*) wait to seize chicks as they emerge.

The 12 species of megapodes differ considerably in their incubation habits. *Macrocephalon*, *Eulipoa*, *M. pritchardii* and some *M. freycinet* simply lay the egg in a Sun-warmed or volcano-warmed hole at the desired temperature and then leave, while *Aepyodius*, *Talegalla*, *Leipoa*, *Alectura*, *M. lapérouse* and some *M. freycinet* remain to tend their compost heap and regulate its temperature. The *M. freycinet* population of the Solomons uses all three heating techniques employed by the whole family and we have no idea whether a given female is programmed to adopt one of these methods or can choose. Among species that tend mounds, the male and female of *Talegalla*, *M. freycinet* and *M. lapérouse* share the work; the male *Leipoa* does most of the work; and the male *Alectura* does all the work and permits the female to lay only when he is satisfied that the temperature is correct. Most megapodes live near (or at the same elevation as) their incubation site. However, *E. wallacei* of the Moluccas lives in the mountains above 1,500m, and the female flies down to a coastal nesting beach only on the night that she is ready to lay^{2,6}.

Most megapodes nest solitarily, but it has long been known that four species nest communally. The most famous social nesting grounds are those at the volcanoes used by *M. pritchardii* and some *M. freycinet* and the beaches used by *Eulipoa* and some *Macrocephalon*. These communal sites are patronized by hundreds of birds. Crome and Brown¹³ recently discovered a more sophisticated and selective communal arrangement in Australian mound-tending *M. freycinet*: pair number 3 permitted the neighbouring pair number 4 to use the mound in pair 3's territory but tolerated no other pair. For a long time the only hint of interspecific mound parasitism was Siebers' discovery of a *Eulipoa* egg laid in a *M. freycinet* mound on the Moluccan island of Buru². However, Dwyer, working in the mountains of New Guinea, has now dug four *M. freycinet* eggs out of a mound

maintained by *Talegalla*, caught *M. freycinet* in the act of digging into the *Talegalla* mound and obtained evidence that *M. freycinet* also uses *Aepyodius arfakianus* mounds and is a habitual mound parasite in Dwyer's study area¹⁴.

The peculiar nesting habits of megapodes have diverted interest from their other interesting characteristics. One such characteristic, shared with cuckoos of genus *Centropus*, is that the flesh of *M. freycinet* and *Talegalla jobiensis* develop a repugnant stench soon after death. Rapid putrefaction may serve an anti-predator function similar to the poisonous or bad-tasting flesh of some insects and is the basis of the following Solomon islanders' tradition: if you want to eat a megapode, you should go to the forest, find the tree where the megapode roosts at night, boil a pot of water under the tree and shoot the megapode when it alights to roost so that it falls into the boiling pot. Only then can one hope to obtain a megapode for cooking.

Among the many unsolved problems posed by megapodes' reproductive biology, I briefly call attention to two. First, Baltin¹¹ has suggested that the main reason why megapodes spend so much time digging in their mounds is to regulate oxygen and carbon dioxide levels around the egg. While Frith's experiments leave no doubt of a temperature-regulating function, the question of why megapode eggs and chicks do not die of O₂ deficit and CO₂ narcosis in the mounds (CO₂ levels nearly eight times atmospheric) poses an obvious challenge to physiologists¹⁵. Second, megapodes resemble most reptiles in numerous aspects of egg biology, such as underground incubation, lengthy incubation at temperatures below those of most other birds, high yolk content, precocious hatched young and no parental care of young. Are megapode nesting habits a primitive inheritance from reptiles, or were the habits derived secondarily from nesting habits of normal birds? In favour of the latter interpretation, at least one other bird, the Egyptian plover *Pluvianus aegyptius*, also buries its eggs and regulates their temperature by a combination of solar heat, body heat, shading and watering¹⁶. □

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Onco gen

Human oncogene locations and chromosome aberrations

from Janet D. Rowley

GREAT excitement has been caused in the past few months by the new links that have been revealed between the study of cellular oncogenes and the analysis of chromosome abnormalities in human tumours. A remarkable concordance between the chromosome location of human cellular oncogenes and the breakpoints involved in chromosome translocations and deletions in various forms of cancer is becoming apparent. The oncogenes *c-mos*¹, *c-myc*¹⁻³ and *c-abl*⁴ have been located at the breakpoints in the 8;21, 8;14 and 9;22 translocations associated with acute myeloblastic leukaemia⁵, Burkitt's lymphoma⁶ and chronic myelogenous leukaemia⁷ respectively, whereas *c-ras*^H is located at the site of the deletion present in patients with the aniridia-Wilms' tumour syndrome⁹.

The figure summarizes the location of oncogenes on specific chromosomes, the nonrandom changes affecting the chromosomes and the diseases in which the karyotypic abnormalities are frequently seen. Karyotypic abnormalities have been known to exist in human tumours for quite some time. Consistent aberrations are found primarily in haematological diseases¹⁰, although they are also seen in tumours of embryonic origin^{9,11}, in meningiomas^{12,13} and in some other cancers^{14,15}. Specific translocations are a

prominent feature of particular types of leukaemia and lymphoma.

Of all the oncogenes, most information is available on *c-myc*; it is found on chromosome 8 in man¹⁻³ and on chromosome 15 in the mouse^{2,16}. The translocation common in Burkitt's lymphoma — involving the long arms of chromosomes 8 and 14 (t(8;14))⁶ — brings about a break in chromosome 14 at band q32 within the locus for the immunoglobulin heavy-chain gene¹⁷. The break seems to occur in different nucleotide sequences in different Burkitt's tumour cell lines; Dalla-Favera *et al.*² reported that the break was in the variable region, but Taub *et al.*³ located it at the switch region of the gene. Both laboratories were able to demonstrate in several cell lines that the *c-myc* gene had been translocated from its normal position on chromosome 8 to a new location adjacent to the immunoglobulin heavy-chain locus on chromosome 14. Single DNA restriction fragments containing both a part of the immunoglobulin gene cluster and the *c-myc* gene were isolated from different Burkitt's cell lines. Dalla-Favera *et al.*² also found DNA homologous to that of the variable region of the immunoglobulin heavy-chain gene on chromosome 8, indicating that the chromosome exchange was reciprocal. The gene rearrangement found in human

Burkitt's lymphoma also occurred in mouse plasmacytomas^{18,19} as has been revealed by several laboratories. This was not unexpected because the most common translocation in plasmacytomas is t(12;15)²⁰⁻²², and in the mouse the immunoglobulin heavy-chain gene is on chromosome 12 (ref.23) and *c-myc* is on chromosome 15 (ref.16).

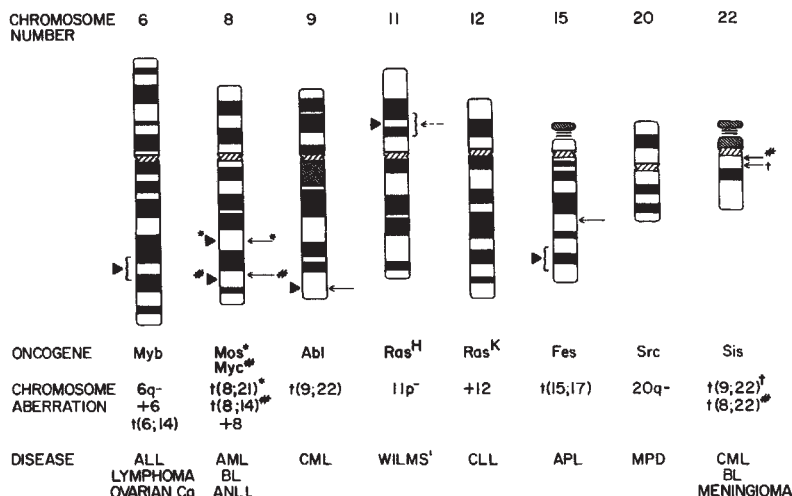
Two other human cellular oncogenes have been mapped to bands consistently involved in translocations. Neel *et al.*¹ have reported that *c-mos* is located at 8q22, the breakpoint of chromosome 8 in the 8;21 translocation seen primarily in acute myeloblastic leukaemia⁵. De Klein *et al.*⁴ have located *c-abl* at 9q34 — the breakpoint of chromosome 9 in the 9;22 translocation which occurs in chronic myelogenous leukaemia (CML)⁷. Moreover, by using somatic cell hybrids, they could show that *c-abl* was translocated to the Philadelphia (22q-) chromosome. This is the first gene normally located on chromosome 9 whose translocation to chromosome 22 has been demonstrated in CML, and thus provides genetic evidence that this translocation also is reciprocal.

A fourth oncogene to be located precisely is *c-ras*^H, to 11p13 (ref.8). This is of particular interest because a deletion of 11p13 as a constitutional chromosome abnormality associated with aniridia predisposes patients to Wilms' tumour⁹. Moreover, a patient with a normal karyotype whose Wilms' tumour cells had a deletion of 11p13 has been described²⁴. The precise location of *c-ras*^H was determined not with the cellular oncogene, but with DNA obtained from transfection experiments. The DNA, derived from a human bladder carcinoma, EJ, had previously been shown to be homologous to that of *c-ras*^H (ref.25). It has been demonstrated with various techniques that *c-ras*^H and *c-ras*^K belong to a closely related oncogene family. *c-ras*^H is found on chromosome 11 (ref.26) and *c-ras*^K is on chromosome 12 (ref.27). The two chromosomes have similar banding patterns as well as genetic homology, and are assumed to have arisen by duplication in the distant past. For example, the gene for lactate dehydrogenase A (LDHA) is on the short arm of chromosome 11 and LDHB is on the short arm of chromosome 12 (ref.28); the location of *ras*^H and *ras*^K on chromosomes 11 and 12 respectively is, therefore, not surprising.

The oncogene *c-myb* has been localized to 6q22-q24 (ref.29), which is in the region of the breakpoints in the long-arm deletion seen in acute lymphoblastic leukaemia (ALL)³⁰. Sixteen of eighteen ALL patients with a 6q- chromosome had a deletion in bands 6q21 to q25 (ref.30). In the translocation involving chromosomes 6 and 14 in ovarian cancer, the break in chromosome 6 is in band 6q21 (ref.31).

Although the evidence summarized so far indicates a close correlation between the breakpoints associated with consistent

Diagram of chromosomes containing known cellular oncogenes; the number is above each chromosome, and the oncogene, karyotypic aberrations and neoplastic diseases associated with these aberrations are indicated below the chromosome. The arrowhead (▶) to the left of each chromosome indicates the band carrying the cellular oncogene; the arrow or arrows to the right of a chromosome identify specific bands involved in consistent translocations (↔) or deletions (←) observed in patients having the disorders listed. *Indicates the *c-mos* and t(8;21); #, *c-myc* and t(8;14) or t(8;22). AML, acute myeloblastic leukaemia; CML, chronic myelogenous leukaemia; APL, acute promyelocytic leukaemia; ANLL, acute nonlymphocytic leukaemia; CLL, chronic lymphocytic leukaemia; MPD, myeloproliferative disorder. p indicates the short arm, and q the long arm of the chromosome. Ref.36 describes the locations of *c-abl* and *c-fes*, and refs 37 and 38 that of *c-src* and *c-sis* respectively.

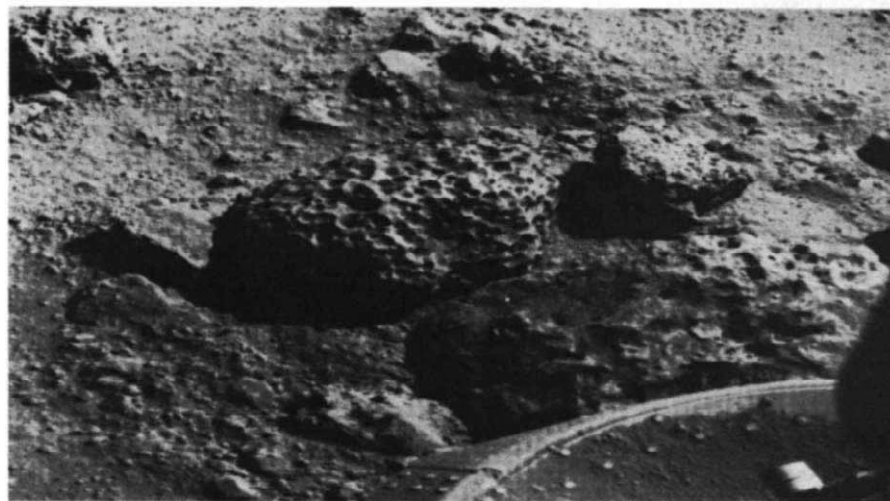


Can honeycomb weathering be ET?

from Peter J. Smith

SOME months ago (*Nature* 298, 121; 1982) I drew attention to George Mustoe's study of honeycomb weathering in the arkosic sandstone of the Chuckanut Formation, Washington state. Honeycomb weathering is the evocative name given to the pattern of small (up to 10 cm) cavities found on the surface of various rock types around the world. The phenomenon is easier to name than to explain, however. After rejecting a number of both plausible and exotic mechanisms put forward for consideration over the past 80 years or so, Mustoe (*Bull. geol. Soc. Am.* 93, 108; 1982) concluded from a detailed study that the honeycomb weathering of the coastal Chuckanut Formation was due to the physical (as opposed to chemical) action of salt crystallization. Salt crystals from spray evidently cause differential erosion, producing cavities that are prevented from growing and coalescing by a covering of algae.

As I said at the time, what goes for the



Chuckanut may or may not go for honeycomb-weathered rocks elsewhere, not least for those at arid inland sites where sources of salt are less conspicuous. What I had in mind were sites elsewhere on Earth. However, J. Garvin of Brown University, Rhode Island, has written to point out that honeycomb-type patterns also occur on rocks beyond the Earth. The accompanying NASA photograph, provided by Garvin, shows pitted rocks at the Viking Lander 2 site (Utopia Planitia) on Mars. The boulder near the centre, which may be basaltic, is about 23 cm long; and the pits in it are typically less than 1 cm long.

The most obvious possibility is that the pits are vesicles — cavities formed by the

trapping of gas bubbles during solidification of the rock. On the other hand, as Garvin *et al.* have previously pointed out (*The Moon and Planets* 24, 355; 1981), pits, flutes and other types of cavity occur so frequently in martian rocks that weathering by wind must be regarded as a highly probable cause. There is even some evidence for solution (water-assisted) erosion. Under the circumstances, Garvin considers it far from impossible that saline frosts at Utopia Planitia (48°N) could produce honeycomb weathering much as it is produced in terrestrial rocks. □

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chromosome translocations and deletions and the bands containing oncogenes, one cannot assume that this relationship is invariant. Two sets of preliminary data suggest that *c-fes* and the 15;17 translocation noted in acute promyelocytic leukaemia (APL)³² may be unrelated. First, *c-fes* appears to be located in band 15q24-25 (ref. 29). Although the breakpoint in chromosome 15 in this translocation has been localized to various bands, including q24-q25, our current interpretation is that it is in q22 (J. Rowley, unpublished). If this breakpoint is correct, then *c-fes* is at some distance from the band involved in the 15;17 translocation. Second, Sheer *et al.*³³ have determined that the 15q⁺ translocation chromosome does not contain *c-fes*, which presumably has been translocated to chromosome 17. Based on the complex variants of the simple translocations seen in leukaemia and lymphoma, I proposed that, in APL, the 15q⁺ chromosome was the critical recombinant³⁴. This prediction has been correct, up to now, in the only two translocations for which data are available; thus, the 14q⁺ chromosome and the Ph¹ chromosome were predicted to be the essential recombinants in Burkitt's lymphoma and CML respectively, and each is the recipient of DNA containing an oncogene.

In the future, we will see the

chromosome localization of the remaining oncogenes, as well as that of the other transforming DNAs that have been identified by transfection assays. The mechanism or mechanisms by which translocations or deletions of chromosomes containing cellular oncogenes are related to malignant transformations are unknown, although there is evidence that malignant transformation is a complex process that is

the result of the interaction of several genes. When the nature of the functional alterations of oncogenes is clarified, the role of chromosome aberrations in cancer, which has been a puzzle since the time of Boveri³⁵, may finally be resolved. □

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Nuclear safeguards

Closing the accountability gap

from David H. Smith

INHERENT in the operation of civilian nuclear reactors is the risk that fissile material may be diverted for the construction of nuclear weapons. The feed material, which in the case of light-water reactors typically contains 2–4 per cent uranium-235, could be surreptitiously diverted and run through an enrichment process to bring the ^{235}U concentration up to the 20 per cent or so minimum needed to make a bomb (though 90 per cent is a more usual concentration for weapons); alternatively, spent fuel, which contains plutonium-239 (produced in the reactor from non-fissile ^{238}U), could be diverted and reprocessed to extract the plutonium.

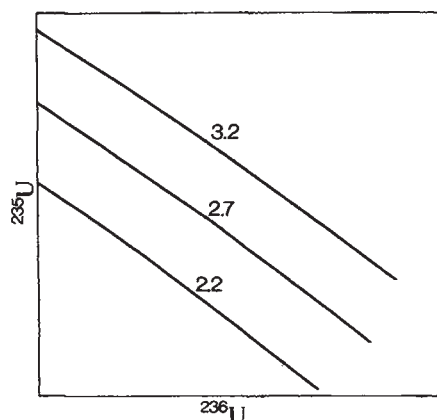
Current inspection procedures of the International Atomic Energy Agency are adequate to prevent diversion of feed material and spent fuel, but offer little assurance that the reactor operator is not removing ^{239}Pu continuously from the reactor and making up the balance with unirradiated uranium.

A new analytical technique recently reported* by Paul Persiani (Argonne National Laboratory) should, however, close this gap.

The isotope correlation methods described by Persiani exploit the fact that, for a given type of reactor, the build-up and decay of the various isotopes are known as functions of feed material, reactor characteristics and length of burn-up. The amount of ^{236}U that accumulates in a given reactor from burning of fuel of a given initial isotopic composition can, for example, be predicted as a function of burn-up time.

A data base must be established for each type of reactor — pressurized water, light water, or liquid-metal fast breeder — by making the requisite isotopic abundance measurements. For the correlation functions, isotopic ratios are chosen such that monotonic approximately linear functions can be obtained when plotted at various burn-up times; a family of such curves is obtained as the isotopic composition of the fuel is varied. Cross-correlation between plots of several different functions for a given sampling provide virtual certainty of the conclusions drawn. Some of the more useful plots would be $^{236}\text{U} \vee ^{235}\text{U}$, $^{235}\text{U} \vee (^{239}\text{Pu})^2$, $(^{239}\text{Pu}^{242}\text{Pu}) / (^{240}\text{Pu})^2 \vee \text{Pu}/\text{U}$, $^{241}\text{Pu}/^{240}\text{Pu} \vee \text{Pu}/\text{U}$ and $(^{241}\text{Pu} + ^{242}\text{Pu}) / ^{240}\text{Pu} \vee \text{Pu}/\text{U}$, with isotopic abundances in weight per cent and Pu/U indicating the weight ratio of these elements in the solution.

As an example of how the technique works, the figure shows plots of ^{235}U versus ^{236}U for three different initial fuel compositions.



^{235}U plotted against ^{236}U for three different initial fuel compositions.

sitions (2.2, 2.7, 3.2 per cent ^{235}U). Different lengths of burn-up yield points falling at different places along whichever line is appropriate. Substitution of unirradiated material will result in a deviation from the expected line through alteration of the ^{236}U content relative to that of ^{235}U .

To effect such a correlation scheme, the fuel must be sampled after removal from the reactor. Spent fuels are highly radioactive and extremely difficult to dissolve and sealed reflux techniques are normally required for complete dissolution^{1,2}. Until

recently, costly and time-consuming chemical separation would be required before sampling but, with the development of the resin bead sampling technique³⁻⁵, which uses anion resin beads to extract uranium and plutonium from 8 M HNO_3 fuel solutions, a simple and easily implemented sampling scheme for these solutions has become available. Subsequent mass spectrometric analysis provides isotopic and quantitative characterization of the two elements.

The combination of isotopic correlation and resin bead sampling-mass spectrometry thus provides a tool for verification of actual reactor operation independent of material balances established by assaying other phases of the nuclear fuel cycle. Diversion of fissile material between shutdown of a reactor and sampling of spent fuel or operation of a reactor to generate weapons-grade plutonium without being detected by inspectors may now become virtually impossible. □

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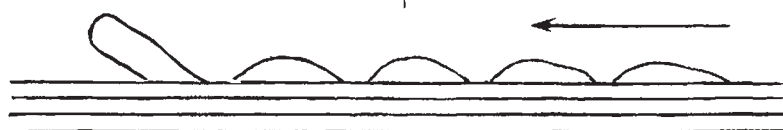
100 years ago

THE SEA SERPENT

BELIEVING it to be desirable that every well-authenticated observation indicating the existence of large sea serpents should be permanently registered, I send you the following particulars.

About three p.m. on Sunday, September 3, 1882, a party of gentlemen and ladies were standing at the northern extremity of Llandudno pier, looking towards the open sea, when an unusual object was observed in the water near to the Little Orme's Head, travelling rapidly westwards towards the Great Orme. It appeared to be just outside the mouth of the bay, and would therefore be about a mile distant from the observers. It was watched for about two minutes, and in that interval it traversed about half the width of the bay, and then suddenly disappeared. The bay is two miles wide, and therefore the object, whatever it was, must have travelled at the rate of thirty miles an

hour. It is estimated to have been fully as long as a large steamer, say 200 feet; the rapidity of its motion was particularly remarked as being greater than that of any ordinary vessel. The colour appeared to be black, and the motion either corkscrew like or snake-like, with vertical undulations. Three of the observers have since made sketches from memory, quite independently of the impression left on their minds, and on comparing these sketches, which slightly varied, they have agreed to sanction the accompanying outline as representing as nearly as possible the object which they saw. The party consisted of W. Barfoot, J. P. of Leicester, F. J. Marlow, solicitor, of Manchester, Mrs. Marlow, and several others. They discard the theories of birds or porpoises as not accounting for this particular phenomenon. F.T. Mott Birstall Hill, Leicester, January 16 From *Nature* 27, 293, 25 January 1883.



*184th National Meeting of the American Chemical Society, Kansas City, Missouri, 12–17 September 1982.

New H_2SO_4 and HSO_3 vapour measurements in the stratosphere—evidence for a volcanic influence

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In situ measurements of stratospheric H_2SO_4 and HSO_3 vapour concentrations using passive chemical ionization mass spectrometry were made in 1982 before and after the dramatic eruptions of the Mexican volcano El Chichón. Substantial increases of the total concentration of these gases over previously measured values were observed, particularly around 25 km altitude where most of the eruption cloud material was deposited. Implications for stratospheric SO_2 -oxidation and nucleation processes are discussed.

THE stratospheric sulphur cycle is important as it leads to the formation of the stratospheric aerosol layer which is composed of H_2SO_4 - H_2O solution droplets and which has the potential to influence the Earth's radiation balance and thereby, at least temporarily, may have an impact on climate¹⁻¹¹. It is thought^{1,12} that sulphur-bearing precursor gases of tropospheric origin, mostly SO_2 and OCS , which reach the stratosphere by large-scale transport or turbulent diffusion, are converted to H_2SO_4 - H_2O aerosol solution droplets. Occasionally, very large amounts of precursor gases, mostly SO_2 , are directly injected into the stratosphere by major volcanic eruptions leading to dramatic increases of the mass of the aerosol layer.

However, the processes involved in the transformation of precursor gases to aerosols are not well understood. In particular, the role of heterogeneous processes is uncertain^{1,13}.

Recently¹⁴⁻¹⁹, new insights into these transformation processes were obtained from *in situ* measurements of intermediary gaseous species, H_2SO_4 and HSO_3 , formed during transformation. These measurements became feasible using passive chemical ionization mass spectrometry (PACIMS). This new method for stratospheric trace gas detection, which was originally introduced by Arnold and his colleagues¹⁴, is based on *in situ* composition measurements of stratospheric ions using balloon-borne mass spectrometers.

We report here on stratospheric H_2SO_4 —and HSO_3 —vapour measurements before and after the big eruptions of the Mexican volcano El Chichón which occurred on 28 March and 4 April 1982 and which are thought²⁰ to be among if not the strongest ones since the eruption of Krakatoa in 1884.

Measurements and results

The PACIMS method used has been described in some detail elsewhere¹⁴⁻¹⁹ and will, therefore, be reviewed here only briefly. Sum concentrations $[\text{H}_2\text{SO}_4 + \text{HSO}_3]$ are inferred by consideration of HSO_4^- formation from NO_3^- and HSO_4^- (rate coefficient k_1) and loss by ion-ion recombination (rate coefficient α). Partial concentrations of H_2SO_4 and HSO_3 are inferred by consideration of H_2SO_4 and HSO_3 clustering to $\text{HSO}_4^-\text{H}_2\text{SO}_4$ (phenomenological binary rate coefficient k_2) and loss of primary and secondary product ions by ion-ion recombination. A common rate coefficient $k_1 = k_2 = 10^{-9} \text{ cm}^3 \text{ s}^{-1}$ and a common α were taken. For α new computer simulation data of Bates²² were used.

Two balloon flights were conducted over southern France (44°N) during daytime. The first flight (flight B21) was on 12 March 1982 and reached an altitude of 34 km. Negative ion data were obtained from one mass spectrometer during the ascent part of the flight between ~22 and 26 km. The second flight (flight B22) took place on 10 June 1982 and reached the same altitude. Here negative ion data were obtained from two mass spectrometers mounted in the same balloon gondola. This measurement (B22) covered the height ranges 22–34 km

(ascent) and 34–26 km (descent). Ascent and descent velocities were ~6 and 1 m s^{-1} , respectively.

Measured $[\text{H}_2\text{SO}_4 + \text{HSO}_3]$, $[\text{H}_2\text{SO}_4]$ and $[\text{HSO}_3]$ values are shown in Fig. 1. The former cover the entire height region from about 22 to 34 km whereas partial concentrations could be obtained only above 27 km. In the B21 measurement only $[\text{H}_2\text{SO}_4 + \text{HSO}_3]$ values (22–26 km; ascent) could be obtained.

For B22 ascent and descent data as well as data obtained from different instruments are in good agreement. It is also found that $[\text{HSO}_3]$ is smaller than $[\text{H}_2\text{SO}_4]$ above ~28 km. Only at the lowest height where HSO_3 could be measured, $[\text{HSO}_3]$ seems to be comparable with $[\text{H}_2\text{SO}_4]$.

The B21 $[\text{H}_2\text{SO}_4 + \text{HSO}_3]$ values are similar to the B22 values. As the error for relative $[\text{H}_2\text{SO}_4 + \text{HSO}_3]$ values is about a factor of 1.5, the error bars of B21 and B22 values are overlapping except for 25 km where the B22 value is larger by a factor of ~5.

Sulphuric acid evaporation region

A comparison of present $[\text{H}_2\text{SO}_4 + \text{HSO}_3]$ and $[\text{H}_2\text{SO}_4]$ values with previous data of our group^{17,18} and with model calculations of Turco *et al.*²³ for $[\text{H}_2\text{SO}_4]$ are shown in Fig. 2. Above about 27 km, the June 1982 data agree well with the June 1980 values. In this region H_2SO_4 is controlled by the equilibrium saturation pressure of H_2SO_4 vapour over the H_2SO_4 - H_2O aerosols which, in turn, depends sensitively on the temperature. Curves 45°S and 45°W¹⁵ give equilibrium saturation concentrations for H_2SO_4 vapour for average summer and winter temperatures at 45° latitude. Curve 45°S matches the June 1980 and 1982 data very well between about 27 and 32 km.

The observed steep increase of $[\text{H}_2\text{SO}_4]$ above 27 km reflects the rise of atmospheric temperatures with increasing height¹⁵⁻¹⁹. Above ~32 km the B22 data become increasingly smaller than curve 45°S, suggesting that all H_2SO_4 is in the gas phase. This implies that the top of the H_2SO_4 - H_2O aerosol layer must be located around 32 km in summer.

Interestingly, the maximum $[\text{H}_2\text{SO}_4]$ of $\sim 2 \times 10^6 \text{ cm}^{-3}$ reached around 34 km is substantially smaller than that predicted by a model considering only a small loss of H_2SO_4 vapour by weak photolysis (curve 2 in Fig. 2). Thus, it seems that there exists a more efficient sink for H_2SO_4 vapour in this region. Potential sinks are stronger photolysis or heterogeneous processes involving 'meteor smoke particles'²³ (curve 3 in Fig. 2). If H_2SO_4 photolysis was comparable with that of HNO_3 and not as assumed by the model with that of HCl , the low $[\text{H}_2\text{SO}_4]$ measured around 34 km could be accounted for.

Removal of H_2SO_4 vapour by 'meteor smoke' is uncertain as these particles are, as yet, purely hypothetical.

The autumn 1981 $[\text{H}_2\text{SO}_4 + \text{HSO}_3]$ values are systematically lower than the June 1980 and 1982 data above 27 km which can readily be explained by lower autumn temperatures giving

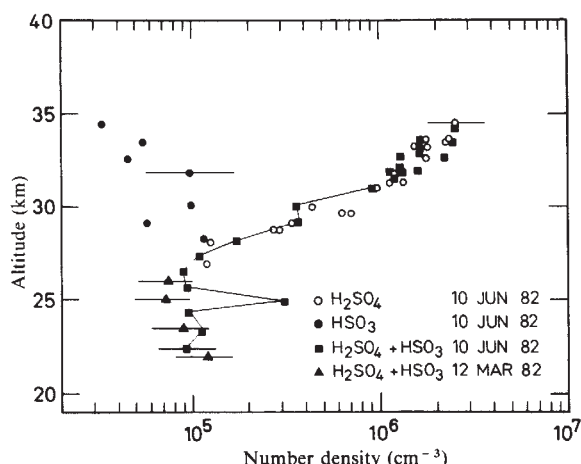
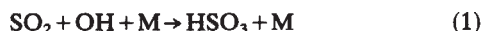


Fig. 1 Concentration of H_2SO_4 and HSO_3 vapours as measured in the balloon flights B21 and B22 on 12 March and 10 June 1982, respectively. A model prediction for HSO_3 of Turco *et al.*²³ is shown for comparison. Typical errors for absolute measured concentrations are indicated.

rise to lower saturation pressures. The autumn data range well between curves 45°S and 45°W.

A comparison of present $[\text{HSO}_3]$ values with those inferred from previous negative ion composition data (autumn 1981) of our group^{17,18} and theoretical model profiles is shown in Fig. 3. Within the error the June 1982 and autumn 1981 data agree reasonably well. They are also similar to the values predicted by the model of Turco *et al.*²³ (curve 1 in Fig. 3) which considers HSO_3 formation by process



(rate coefficient¹³: 3×10^{-31} (300/ T)^{2.9} $\text{M cm}^6 \text{s}^{-1}$) and HSO_3 loss by process



(rate coefficient unknown; an upper limit²³ of $10^{-11} \text{ cm}^3 \text{s}^{-1}$ was assumed) followed by



(rate coefficient²³: $9.1 \times 10^{-13} \text{ cm}^3 \text{s}^{-1}$). However, HSO_3 vapour may also be removed heterogeneously by interaction with H_2SO_4 - H_2O aerosols as proposed by Arnold *et al.*¹⁷. Assuming the latter process as the only HSO_3 sink one obtains curve 2 in Fig. 3. Here a sticking or reaction probability of 1 was assumed and a typical lifetime, t , for a molecule against collision with aerosols²³ was taken.

Above ~30 km HSO_3 loss through the OH reaction (2) seems to be faster than heterogeneous removal whereas below this height the reverse seems to be true. In view of the substantial errors of the model values the measured data and curves 1 and 2 are in rough agreement.

Below ~30 km, however, the $[\text{H}_2\text{SO}_4 + \text{HSO}_3]$ values measured, for example, in autumn 1981 become distinctly smaller than curve 1 and even smaller than curve 2.

Thus, it seems that either the HSO_3 sink is more efficient than reaction (2) or that the HSO_3 source is weaker or both. This may support the view of heterogeneous HSO_3 removal.

Volcanic cloud region

Below 27 km the June 1982 $[\text{H}_2\text{SO}_4 + \text{HSO}_3]$ data are larger than the autumn 1981¹⁸ and June 1980¹⁵ data which are particularly true around 25 km. The March 1982 data seem to range in between. In view of an error of $\pm 50\%$ estimated for relative $[\text{H}_2\text{SO}_4 + \text{HSO}_3]$ values, the differences seem to be significant although error bars are overlapping except for the 25-km value of June 1982.

Because both HSO_3 and H_2SO_4 vapours are only short-lived around 25 km (lifetime <1 day) and as they are probably removed by heterogeneous processes, the increase of $[\text{H}_2\text{SO}_4 +$

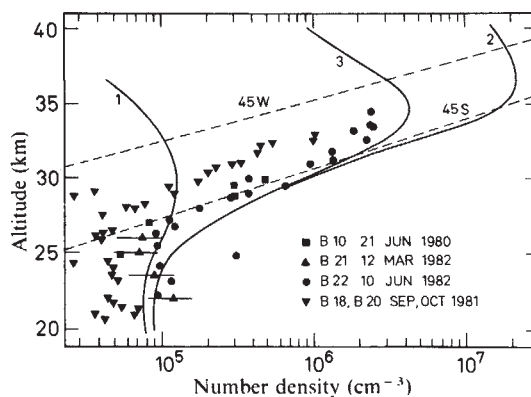


Fig. 2 Comparison of presently measured concentrations $[\text{H}_2\text{SO}_4 + \text{HSO}_3]$ and $[\text{H}_2\text{SO}_4]$ with previous measurements (June 1980 and autumn 1981) of our group^{15,16}. Model calculations for H_2SO_4 vapour concentrations of Turco *et al.*²³ (curve 1, excluding H_2SO_4 evaporation from aerosols; curve 2, including H_2SO_4 evaporation; curve 3, including heterogeneous removal of H_2SO_4 vapour by 'meteor smoke') are also given. Curves 45°S and 45°N give equilibrium saturation concentrations of H_2SO_4 vapour over H_2SO_4 - H_2O aerosols for average summer and winter temperatures at 45° latitude¹⁵.

$\text{HSO}_3]$ observed around 25 km in June 1982 suggests a marked increase of the production P of HSO_3 and/or H_2SO_4 .

It seems possible that the enhanced P was due to an injection of sulphur-bearing precursor gases (mostly SO_2) and possibly also H_2O by the dramatic eruptions of volcano El Chichón which occurred on 28 March and 3 April 1982 in Mexico. From optical observations of aerosols made over Hawaii in May 1982 the eruption cloud was found to extend over the height range 20–32 km, being densest around 26 km. It was also found (Reiter, personal communication) that the cloud had already reached central Europe by May.

The correspondence of the densest part of the aerosol cloud and our peak $[\text{H}_2\text{SO}_4 + \text{HSO}_3]$ value around 25–26 km supports a volcanic cause of the observed June 1982 enhancement of $[\text{H}_2\text{SO}_4 + \text{HSO}_3]$.

It cannot be excluded that even the autumn 1981 and particularly the March 1982 data are under some volcanic influence. Major volcanic eruptions occurred on 18 May 1980 (St Helens), on 28 April 1981 (Alaid) and January 1982 (unidentified volcano).

Due to the large increase in aerosol mass the total aerosol surface area density A also must have increased and therefore the lifetime, t , of H_2SO_4 and probably also HSO_3 vapour molecules against heterogeneous removal must have decreased markedly.

Assuming HSO_3 formation through the key gas-phase SO_2 oxidation process (1), HSO_3 loss through heterogeneous removal and conversion to H_2SO_4 through reaction (2) and (3), and heterogeneous removal of H_2SO_4 , a steady-state approximation yields

$$P = [\text{HSO}_3 + \text{H}_2\text{SO}_4]t^{-1} \quad (4)$$

with $P = [\text{SO}_2]k[\text{OH}]$.

Taking an upper limit for t of $5 \times 10^4 \text{ s}$ (typical for undisturbed conditions), a lower limit for P of $\sim 6 \text{ cm}^{-3} \text{s}^{-1}$ is estimated from the measured $[\text{H}_2\text{SO}_4 + \text{HSO}_3] = 3.1 \times 10^5 \text{ cm}^{-3}$ at 25 km. As our ion measurements do not show any significant depletion of ions in the 25-km cloud, t must be larger than the ion-ion recombination lifetime being $\sim 700 \text{ s}$. Taking the latter value as the lower limit to t , one obtains an upper limit to P of $\sim 440 \text{ cm}^{-3} \text{s}^{-1}$ at 25 km. Following the same approach, a P of about $1 \text{ cm}^{-3} \text{s}^{-1}$ is inferred from the autumn 1981 data at 25 km. By comparison, the P predicted by theoretical models is $\sim 3 \text{ cm}^{-3} \text{s}^{-1}$ if $[\text{SO}_2] = 5 \times 10^6 \text{ cm}^{-3}$, $[\text{OH}] = 2 \times 10^6 \text{ cm}^{-3}$ and $k = 3.2 \times 10^{-13} \text{ cm}^3 \text{s}^{-1}$ are taken.

Preliminary estimates of the increase of A based on optical depth measurements suggest that the increase may be of the order of 10. If correct, this would imply a 10-fold smaller t and

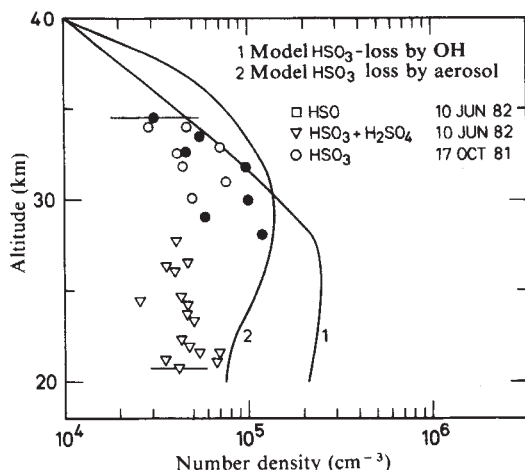


Fig. 3 Comparison of $[\text{HSO}_3]$ and $[\text{H}_2\text{SO}_4 + \text{HSO}_3]$ (below 28 km) measured in June 1982 and autumn 1981. Theoretical model calculations of Turco *et al.*²³ (curve 1) assuming HSO_3 loss by OH reaction are also shown. Curve 2 is a model prediction for HSO_3 loss through heterogeneous removal taking an HSO_3 production as used for curve 1 and molecule-aerosol collision frequency as given by Turco *et al.*¹.

consequently a 10-fold larger P than our estimated lower limit of $6 \text{ cm}^{-3} \text{ s}^{-1}$. Taking this probable P of $60 \text{ cm}^{-3} \text{ s}^{-1}$ it would require only about 15–30 days to double the mass of stratospheric $\text{H}_2\text{SO}_4\text{--H}_2\text{O}$ aerosols around 25 km. The inferred increase of P is probably due to an increase of $[\text{SO}_2]$ and possibly also $[\text{OH}]$. The latter may result from injection of water vapour¹³.

The lifetime of SO_2 against process (1), in undisturbed conditions, is about 10 days at around 25 km. If $[\text{OH}]$ was enhanced this lifetime would be even smaller. As our June 1982 measurement took place about 2 months after the El Chichón eruptions, the initial $[\text{SO}_2]$ in the early evolutionary phase of the eruption cloud must have been much larger than in June 1982. Alternatively, it may be considered that a depletion of OH may have occurred³ due to SO_2 oxidation which in turn would have increased the SO_2 lifetime.

In any case our measurements suggest that efficient gas phase SO_2 oxidation occurs and that the reservoir of excess sulphur-bearing precursor gases injected by the eruptions of El Chichón was not exhausted even 2 months after the eruptions.

Implications for nucleation and aerosol growth

It has been shown recently²⁴ that ion nucleation is a potential source of stratospheric condensation nuclei. However, it was also pointed out that severe kinetic limitations exist. They arise from usually low abundances of condensable vapours in the region where ion nucleation is thermodynamically favourable (supersaturation ratio $S \geq 4\text{--}6$).

In the dense cloud at 25 km kinetic limitations are less severe and therefore a strong enhancement of ion nucleation rates over normal values should have occurred. The estimated ion nucleation rate is $\sim 10^{-2}\text{--}10^{-1} \text{ cm}^{-3} \text{ s}^{-1}$ which greatly exceeds the background values. Thus, a concentration of condensation nuclei $[\text{CN}]$ of the order of $10^3\text{--}10^4 \text{ cm}^{-3}$ could be built up within only 1 day, the typical lifetime against heterogeneous removal involving large aerosols. Mutual CN coagulation

should become efficient only for $[\text{CN}] > 10^5 \text{ cm}^{-3}$, a value which may not be reached due to CN attachment to larger aerosols.

Aerosol growth by H_2SO_4 and/or HSO_3 condensation should also be enhanced in the 25-km cloud observed in June 1982. If HSO_3 vapour undergoes heterogeneous removal as discussed above, the rate of gas-to-particle conversion should be equal to $[\text{H}_2\text{SO}_4 + \text{HSO}_3]t^{-1}$. As $[\text{H}_2\text{SO}_4 + \text{HSO}_3]$ is greatly enhanced in the 25-km cloud, the time scale for condensational growth of newly formed very small particles becomes smaller compared with their lifetime against attachment to large particles. Growth, however, increases the lifetime of small particles against attachment to large particles and coagulation as the average thermal velocity of small particles increases.

Consequently, the aerosol size distribution should distinctly change in the 25-km cloud. Generally, the fraction of small particles should increase.

Conclusions

The measured concentrations of H_2SO_4 and HSO_3 vapours have important implications for sulphur oxidation and aerosol processes in the stratosphere. In the height region above ~ 27 km $[\text{H}_2\text{SO}_4]$ is controlled largely by temperature which determines the equilibrium saturation pressure of H_2SO_4 vapour over the stratospheric $\text{H}_2\text{SO}_4\text{--H}_2\text{O}$ aerosol solution droplets. Above ~ 32 km $[\text{H}_2\text{SO}_4]$ becomes increasingly smaller than the equilibrium saturation values suggesting that all H_2SO_4 is in the gas phase. Thus, a sharp upper boundary of the $\text{H}_2\text{SO}_4\text{--H}_2\text{O}$ aerosol layer must exist around 32 km in summer.

Maximum sulphuric acid vapour concentrations of $\sim 2 \times 10^6 \text{ cm}^{-3}$ observed at 34 km are about 10 times smaller than those predicted by theoretical models. Probably this reflects that a more efficient sink for H_2SO_4 vapour is operative at these altitudes. Potential candidates are photolysis and heterogeneous processes involving 'meteor smoke'.

Below ~ 27 km, where H_2SO_4 evaporation from aerosols is unimportant, a marked increase of $[\text{H}_2\text{SO}_4 + \text{HSO}_3]$ from autumn 1981 to June 1982 has been observed. It is particularly pronounced at 25 km where a thin cloud of H_2SO_4 and/or HSO_3 vapours has been detected in June 1982. The height of this cloud coincides with the height of an aerosol cloud detected by Lidar and by *in situ* observations, and originating from the dramatic eruption of the Mexican volcano El Chichón. Therefore it is almost certain that the observed H_2SO_4 and/or HSO_3 vapour cloud is also due to this volcanic eruption.

Our observation suggests that gas-phase SO_2 oxidation was markedly enhanced about 2 months after the El Chichón eruptions. This implies that the reservoir of volcanic sulphur-bearing precursor gases in the 25-km cloud was still not exhausted and that $\text{H}_2\text{SO}_4\text{--H}_2\text{O}$ aerosol formation through gas-phase SO_2 oxidation was still operative with large efficiency.

Future measurements of H_2SO_4 and HSO_3 vapour abundances may elucidate the later evolutionary stages of the stratospheric eruption cloud of El Chichón. It would be desirable to coordinate such measurements with Lidar and accompanying *in situ* observations of aerosols.

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Rearranged mitochondrial genes in the yeast nuclear genome

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We have found a contiguous DNA sequence in the yeast nuclear genome with extensive homology to non-contiguous yeast mitochondrial DNA sequences. Closely linked to this nuclear sequence in some, but not all, yeast strains is a tandem pair of transposable (Ty) elements. Certain features of the content and organization of this nuclear DNA sequence suggest that it may have originated from petite mitochondrial DNA which integrated into the nuclear genome.

MITOCHONDRIAL genomes contribute a limited but essential number of gene products to the biogenesis of organelles that are competent to carry out oxidative phosphorylation (reviewed in refs 1–3). Comparative analysis of mitochondrial genomes shows that, although the size of mitochondrial DNA (mtDNA) differs significantly among species, most of the major and well characterized gene products are the same. The order of these conserved genes may differ from one species to another. Their internal organization may also differ, even within strains of the same species. In addition, genes have been found which are unique to mitochondrial genomes of a given species or group of organisms. For the most part, these genes encode products whose functions are at present speculative or simply unknown.

Three classes of mitochondrial genes have now been identified: (1) All mitochondrial genomes encode RNA components of the mitochondrial protein synthetic apparatus (2 rRNAs and ~25 tRNAs). In addition, yeast^{4–6} and *Neurospora*^{7,8}, and probably *Paramecium*⁹, *Tetrahymena*¹⁰ and *Zea mays*¹¹ contain one protein of the small mitochondrial ribosomal subunit which is translated intramitochondrially. In yeast, the structural gene for this ribosome-associated protein, called *var1*, has recently been identified on mtDNA¹². In these other organisms, the gene for the mitochondrially translated ribosomal protein is presumed to be on mtDNA, although this has

not been demonstrated. No such mitochondrially translated ribosome-associated protein has been identified to date in mammals. (2) mtDNAs encode polypeptide species which are part of the electron transport and ATP synthetase complexes (the three largest subunits of cytochrome oxidase, cytochrome *b* and one or two subunits of the oligomycin-sensitive ATPase). (3) Unassigned reading frames (URFs) have been found which comprise most of the genic differences among mitochondrial genomes. Eight URFs greater than ~250 base pairs (bp) long are found on human, mouse and bovine mtDNA^{13–15}. Although the putative protein products encoded by these URFs have not been identified, it is noteworthy that human mitochondria apparently synthesize ~26 discrete polypeptide species¹⁶. Although the function and genetic origin of the majority of these proteins are unknown, some may well be products of the URF genes.

In the yeast mitochondrial genome, most of the URFs are found within introns of known mosaic genes. Genetic and biochemical studies suggest that two of these URFs, located within introns 2 and 4 of the cytochrome *b* 'long' gene, encode novel *trans*-acting RNA splicing factors or 'maturases'^{17–19}.

The existence of these differences among mitochondrial genetic systems and their utilization of variations of the universal genetic code^{20–23} raise two important and related ques-

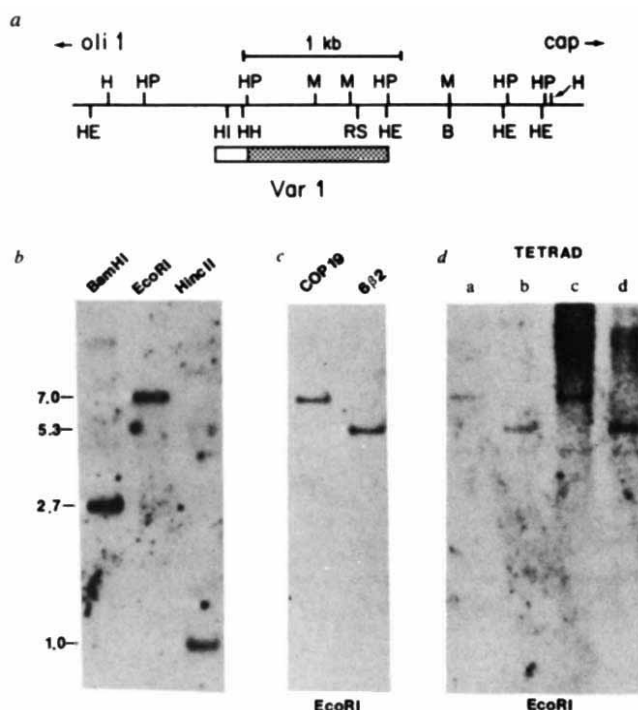


Fig. 1 Hybridization of *var1* DNA to yeast nuclear DNA. **a**, Restriction endonuclease map of the *var1* gene from COP161. **b**, Results of hybridization of pBV3-1 to DNA (10 µg lane) from a ρ^+ derivative of strain COP19 digested with the enzymes indicated. **c**, Results of hybridization of pBV3-1 to *Eco*I-digested DNA from ρ^+ derivatives following a cross between COP19 ρ^+ (α ade⁻lys⁻) and 6 β 2 ρ^+ (a leu⁻trp⁻). **d**, Hybridization results for ρ^+ derivatives of the progeny of the four spores from one tetrad. **Methods:** DNA was isolated from ρ^+ strains of *S. cerevisiae* essentially according to the procedure of Hudspeth *et al.*⁵¹. Restriction endonuclease-digested DNA was fractionated by electrophoresis through 1% agarose gels, transferred to nitrocellulose filters and hybridized with a ³²P-labelled probe containing DNA from the yeast mitochondrial *var1* gene. **a** Shows the cleavage sites for *Hinc*II (H), *Hae*III (HE), *Hpa*II (HP), *Hinf*I (HI), *Hha*I (HH), *Mbo*I (M), *Rsa*I (RS) and *Bam*HI (B). The boxed region below the map identifies the coding region for the *var1* protein. The 0.9-kb *Hpa*II fragment which contains 75% of the coding sequence (shown as the stippled area) was isolated from a *var1*-containing petite strain (A17-10)³³, ligated into the *Cl*aI site of pBR322, and the resulting plasmid (pBV3-1) was used as a probe in the hybridizations of **b**–**d**. Hybridizations were carried out at 5°C in 6×SSC, 1×Denhardt solution and 0.5% SDS. In **b** fragment sizes in kilobase pairs shown on the side were calculated using the yeast ribosomal DNA *Eco*RI and *Hind*III fragments as standards. The cross between COP19 ρ^+ (α ade⁻lys⁻) and 6 β 2 ρ^+ (a leu⁻trp⁻) was made by standard procedures. The resulting diploid progeny were sporulated and three tetrads were dissected and analysed. In all three, the nuclear markers, *ade*, *lys*, *leu* and *trp* showed 2:2 segregation.

tions concerning the origin and evolutionary path of mitochondrial genomes: (1) to what extent has there been interchange of genetic information between the nuclear and mitochondrial genomes, and (2) what has determined the present distribution between the mitochondrial and nuclear genomes, of those genes encoding mitochondrial constituents?

Particularly relevant to these questions is the striking observation that even within the Ascomycetes, there is at least one difference in the genomic location of a gene encoding a mitochondrial protein, the ATPase proteolipid: this gene is located on mtDNA in yeast and in the nuclear genome of *Neurospora*^{24,25}. In the latter, the ATPase proteolipid is synthesized in the cytoplasm and imported post-translationally into the mitochondria. An important implication of these findings is that within the recent evolutionary past, at least for Ascomycetes, there was a transfer of the ATPase proteolipid gene, either from the nuclear to the mitochondrial genome, or vice versa. Although recent evidence suggests that *Neurospora* mtDNA contains a gene sequence with modest homology (~50%) to the nuclear ATPase proteolipid gene²⁶, it is not possible from these observations to deduce whether in fact there has been a gene transfer and in which direction such a transfer might have occurred. Here we report results with yeast which strongly suggest that transfer of DNA sequences between the nuclear and mitochondrial genome has indeed occurred and that the direction of such a transfer was from the mitochondrial to the nuclear genomes.

Using a probe consisting of a portion of the yeast mitochondrial *var1* gene cloned into pBR322, we have discovered a DNA sequence in the yeast nuclear genome homologous to a part of the *var1* gene. Unexpectedly, we have found that immediately adjoining the 5' end of this nuclear *var1* DNA sequence is a sequence homologous to the 3' end of the cyto-

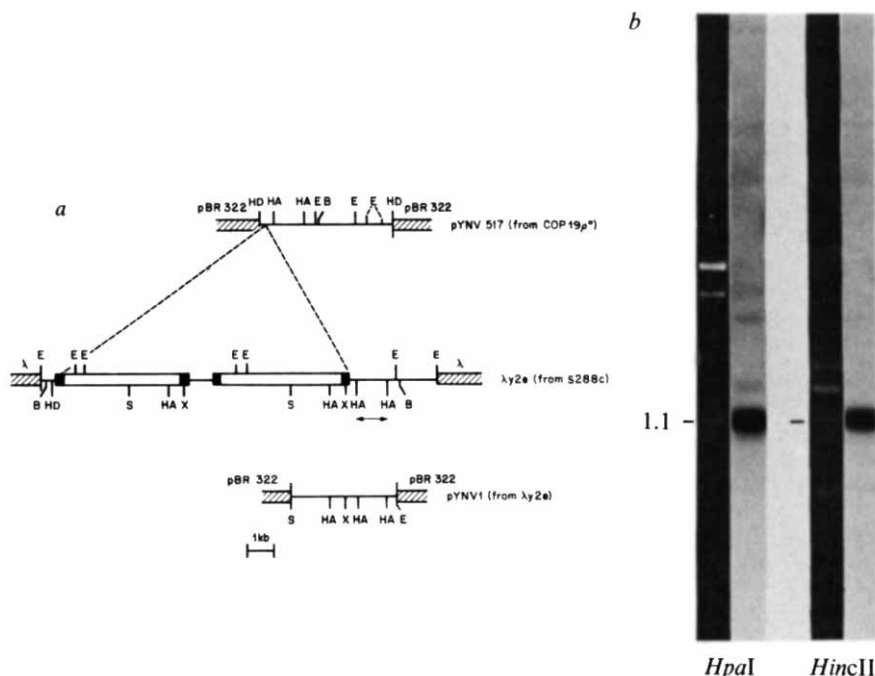
chrome *b* gene (*cob/box*), and 5' to that, a sequence homologous to a portion of an *ori/rep* sequence (sequences located on yeast mtDNA which have been implicated to function in mtDNA replication)^{27,28}. These results lead us to propose a model for the transfer of mtDNA sequences to the yeast nucleus whereby a rearranged DNA fragment, characteristic of petite mtDNA, has become stably integrated into the yeast nuclear genome.

Hybridization of a *var1* probe to nuclear DNA

To screen the yeast nuclear genome for sequences which might be homologous to *var1* DNA, we have used as a probe a recombinant plasmid DNA which contains a 0.9-kilobase (kb) *Hpa*II fragment of *var1* DNA (obtained from a *var1*[40.0] allele) inserted into the *Cl*aI site of pBR322 (Fig. 1a). This insert contains ~75% of the *var1* protein coding sequence and extends 18 bp past the translation terminator (see ref. 12). Hybridization of this *var1* probe to a Southern blot of yeast nuclear DNA (isolated from strain COP19) digested with *Bam*HI, *Eco*RI and *Hinc*II shows the presence of a major hybridizing band at about 2.7, 7.0 and 1.0 kb, respectively (Fig. 1b). Although the yeast nuclear DNA was prepared from a petite derivative of COP19 in which we could detect no mtDNA (ρ^0), there remained the possibility that the DNA fragment detected with homology to the *var1* probe was not a nuclear DNA sequence but rather some highly rearranged, occult piece of mtDNA retained by this petite. To test this possibility, we took advantage of the fact that the *Eco*RI fragment hybridizing to the *var1* probe shows a strain-dependent size polymorphism, illustrated here for two ρ^0 derivatives of strains COP19 and 6 β 2 (Fig. 1c). (Of five independently derived strains we have examined thus far, two contained the 'short' 5.3-kb *Eco*RI

Fig. 2 Cloned nuclear fragments containing a region of homology to *var1* DNA. *a*, Restriction map of a 13.4-kb insert of the *var1* probe λ y2e. *b*, Results of hybridization of λ y2e following digestion with *Hpa*I and *Hinc*II.

Methods: A clone bank⁵² consisting of partially *Eco*RI-digested DNA from *S. cerevisiae* S288C ligated into the *Eco*RI site of λ Charon 4 (obtained from D. Finkelstein) was screened for the presence of sequences homologous to the *var1* probe pBV3-1 (see Fig. 1 legend). Phage were plated at a density of ~1,000 plaques per plate on 10 plates and transferred to nitrocellulose filters. Hybridization with the pBV3-1 probe was carried out in 30% formamide at 42°C. Of 20 positive plaques, 4 were subcloned and found to be identical. The map of one (λ y2e) shows cleavage sites for the enzymes *Eco*RI (E), *Bam*HI (B), *Hind*III (HD), *Sal*I (S), *Hpa*I (HA) and *Xho*I (X) (*a*). The cross-hatched bars represent the DNA adjacent to the inserted yeast DNA. The presumed locations of two Ty elements are indicated as unshaded boxes with flanking shaded areas representing δ sequences²⁹. The exact location of these sequences is unclear, with the exception of the rightmost δ sequence whose location has been confirmed by DNA sequencing. It is not certain whether the two Ty elements are adjoining or whether there are other DNA sequences separating them as shown in the figure. λ y2e DNA was digested with *Hinc*II and with *Hpa*I, the fragments from each of the digests were separated on a 1% agarose gel, transferred to nitrocellulose and hybridized with ³²P-labelled pBV3-1. The result (*b*) shows that the region of homology to *var1* is contained within a 1.1-kb *Hinc*II-*Hpa*I fragment (the recognition sequence for *Hpa*I is also recognized by *Hinc*II), whose location is shown by the double arrow under the λ y2e map. The *Sal*I-*Eco*RI fragment from λ y2e containing the region of homology to *var1* was subcloned by ligation to the large *Sal*I-*Eco*RI fragment of pBR322 to form the plasmid pYNV-1, shown below the map of λ y2e. The plasmid pYNV517, shown at the top, was obtained by ligating *Hind*III-digested DNA from COP19 ρ^0 into the *Hind*III site of pBR322 and screening for homology by using the 1.1-kb *Hpa*I fragment from pYNV1 as a probe. The dotted lines show the position at which the Ty elements, missing in COP19, have been inserted in S288C. The 1.1-kb *Hpa*I fragments of pYNV1 and pYNV517 have identical restriction maps and DNA sequencing confirms that the sequences extending to the left of the *Hpa*I sites are identical up to the beginning of the δ sequence in pYNV1, and that the δ sequence is missing in pYNV517.



fragment.) Thus, a genetic analysis could be carried out to determine the meiotic segregation pattern of the polymorphic *EcoRI* fragment.

Strains COP19 and 6 β 2 were crossed, and randomly selected diploid clones were sporulated; three tetrads were dissected, nuclear genetic markers scored and total DNA isolated from each of the four meiotic products of each tetrad. Results of hybridization of the *var1* probe to an *EcoRI* digest of DNA from one such tetrad show clearly that the *EcoRI* polymorphism segregates 2:2, as expected for a single nuclear locus (Fig. 1d); the results with the other two tetrads analysed were identical (data not shown). Additional evidence that the *var1* probe is detecting a nuclear DNA sequence is obtained from the following. To eliminate a background of mtDNA sequences which would hybridize to the *var1* probe and possibly obscure the results, cultures of each of the meiotic products were treated extensively with ethidium bromide to convert the cells to ρ^0 petites and subcloned before further culturing and isolation of nuclear DNA. In spite of this regime, however, the same DNA fragments homologous to the *var1* probe persist (Fig. 1d).

Isolation of a polymorphic nuclear DNA fragment homologous to *var1* mtDNA

We have used the pBR322-*var1* probe to screen a library of partial *EcoRI* fragments of yeast DNA inserted into λ Charon 4 (provided by D. Finkelstein). This library was constructed from a strain (S288C) containing the 5.3-kb *EcoRI* nuclear DNA fragment homologous to *var1* mtDNA (see Fig. 1). One clone hybridizing with the *var1* probe, designated λ y2e, was isolated which contained a 13.4-kb insert. A restriction map

of that insert is shown in Fig. 2a. To locate the sequence homologous to *var1*, the *var1* probe was hybridized to *HpaI* and *HincII* digests of λ y2e DNA. The results of these hybridization experiments (Fig. 2b) locate the *var1* homology uniquely to a 1.1-kb *HpaI* (*HincII*) fragment (Fig. 2b). For further analysis, a 3.7-kb *SalI*-*EcoRI* sub-fragment of the insert which includes the 1.1-kb *HpaI* segment, was cloned into pBR322. This clone has been designated pYNV1 (Fig. 2a).

Ty elements account for the length polymorphism of the nuclear DNA fragment homologous to *var1*

The restriction map of λ y2e shows that the 13.4-kb insert contains a direct repeat of a 5.4-kb unit of DNA (Fig. 2a). This repeating unit is located to the left of, but does not include, the 1.1-kb *HpaI* fragment homologous to *var1*. The 5.3-kb *EcoRI* fragment of nuclear DNA which hybridizes to *var1* includes the 1.1-kb *HpaI* fragment and extends to the left into the repeating unit. To determine the basis of the variation in the size of this *EcoRI* fragment, a *HindIII* fragment of nuclear DNA homologous to *var1* was cloned from a ρ^0 derivative of COP19, into pBR322. The *HindIII* insert within this plasmid (designated pYNV517) includes the 1.1-kb *HpaI* fragment homologous to *var1*. A comparison of the restriction maps of the DNA inserts in pYNV517 and λ y2e shows an exact correspondence, with the exception of a 10.8-kb insertion (corresponding to the two repeated units) located to the right of the leftmost *HindIII* site in λ y2e (Fig. 2a).

The size and restriction map of the repeated unit of DNA in λ y2e are similar to those of yeast transposable elements (Ty), of which 30-40 similar but not identical copies are dispersed throughout the yeast nuclear genome²⁹. Each Ty element is ~5-6 kb long and contains a terminal repeat of ~350 bp called δ (ref. 29). Indeed, when λ y2e DNA is hybridized to *BamHI*, *EcoRI* or *HincII* digests of yeast nuclear DNA, a large number of hybridizing bands are detected (Fig. 3a). Conclusive evidence that the λ y2e insert repeats are Ty elements was obtained as follows. (1) Two recombinant plasmids containing known Ty inserts (provided by F. Sherman) were hybridized to restriction digests of λ y2e DNA. Both plasmids, one containing a complete Ty repeat (pAA47) and the other which includes δ sequences but no internal Ty sequences (pAA46), hybridize strongly to λ y2e DNA (Fig. 3b). (2) Determination of the nucleotide sequence of the region to the left of the 1.1-kb *HpaI* fragment in λ y2e (Fig. 2a) revealed a sequence beginning 170 bp from the *HpaI* site which is almost identical to known δ sequences^{30,31}, whereas no such sequence was detected in the corresponding region of pYNV517 DNA (data not shown). Thus, we conclude that the size polymorphism for the *EcoRI* fragment containing sequences homologous to *var1* mtDNA is due to the presence or absence of a pair of Ty elements. The lack of δ sequences adjacent to the 1.1-kb *HpaI* fragment of the insert in pYNV517 suggests also that Ty elements were not 'lost' from this region by recombination between δ sequences, because such an event would result in the retention of a δ sequence in the target DNA³⁰.

Additional homology to mtDNA

Before sequencing the relevant regions of the cloned nuclear DNA to determine precisely the extent of *var1* homology, we wished to know if other mtDNA sequences, in addition to *var1*, were present in the nuclear DNA clones. To examine this possibility, pYNV517 DNA was hybridized to Southern blots of *HincII* and *EcoRI* digests of wild-type mtDNA from strain COP19. (As the mtDNA preparations are contaminated with some nuclear DNA, it was not feasible to use λ y2e DNA as a probe because of the large number of hybridizing fragments expected due to the Ty elements.)

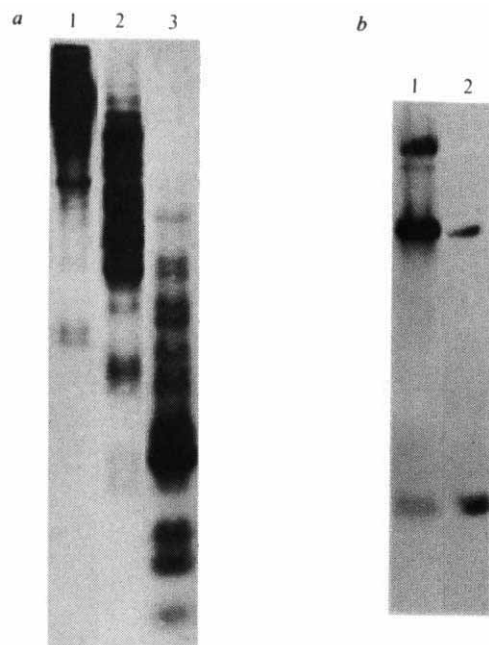


Fig. 3 Identification of Ty sequences in the cloned nuclear fragment. In *a*, DNA from COP19 ρ^0 was digested with *BamHI* (lane 1), *EcoRI* (lane 2) or *HincII* (lane 3), fractionated by electrophoresis on a 1% agarose gel, transferred to nitrocellulose and hybridized with ³²P-labelled λ y2e DNA. *b* Shows the results when λ y2e DNA is digested with *HpaI* and hybridized with ³²P-labelled DNA from either pAA47, a plasmid containing a complete repeat of the yeast Ty element (lane 1), or pAA46, a plasmid containing yeast δ sequences (lane 2). Hybridizations were carried out in 5 $\times$ SSC, 50% formamide, 20 mM NaPO₄ pH 6.5, 1 $\times$ Denhardt solution and 0.2% SDS at 42 °C.

Identification of nuclear DNA sequences homologous to *var1*, *cob/box* and *ori/rep* mtDNA

The nuclear DNA sequence shown in Fig. 5 is 81% A+T with one 26-bp segment which is 73% G+C. This feature—short G+C-rich sequences embedded within long stretches of A+T-rich DNA—is typical of the general organization of the yeast mitochondrial genome in which A+T-rich stretches are frequently interspersed with short (20–60 bp) G+C-rich clusters³⁵. The region of this nuclear DNA sequence with homology to *var1* extends for about 115 bp and is >85% homologous to a segment within the middle of the *var1* gene located between nucleotides 518 and 633 of the 1,188-bp coding sequence of the *var1*[40.0] allele (ref. 12).

The nucleotide sequence shown in Fig. 5 also confirms that the hybridization of the cloned nuclear DNA to *Hinc*II fragment 8 and *Eco*RI fragment 6 of wild-type mtDNA is due to sequence homology to the 3' end of *cob/box* (see ref. 36). This region of homology (>80%) extends for about 123 bp and includes the last 64 bp of exon of *cob/box* (the last exon of the 'long' form of the *cob/box* gene) and proceeds 61 bp beyond the TAA translation terminator. Like the *var1* homology, the sequence differences include some mismatching and a few insertions and deletions.

Notably, there is significant overlap between the sequences homologous to *var1* and *cob/box*, corresponding to a ~67% nucleotide homology between the respective mitochondrial *var1* and *cob/box* sequences (Fig. 5). Such homology between these genes provides a simple mechanism for the apparent fusion of the *cob/box* and *var1* sequences (see below).

Immediately adjacent to the 5' end of the nuclear sequence homologous to *cob/box* exon 6 is the 26-bp G+C-rich cluster referred to above. With the exception of two deleted base pairs, 19 bp of this sequence are homologous to a portion of a G+C-rich cluster in intron 5 of *cob/box* (referring again to the 'long' form of *cob/box*), located ~540 bp from the 3' end of the gene.

Examination of a number of spontaneous petite DNAs has shown that highly suppressive petites arising from different regions of the wild-type mitochondrial genome share a common sequence of some 300 bp which has been implicated in mtDNA replication, possibly as the replication origin^{24,27,28}. Wild-type mtDNA contains three to seven similar but not identical copies of this sequence, referred to as *ori* or *rep* sequences. Contained within *ori/rep* is a 29-bp palindromic A+T-rich sequence flanked by two short G+C clusters. This sequence is capable of forming a hairpin loop and, by analogy with an origin of replication of HeLa mtDNA, it has been suggested that it may be an important element in the origin of replication of yeast mtDNA³⁷. As shown in Fig. 5, adjacent to the 5' end of the sequence homologous to *cob/box* intron 5 is a 29-bp sequence of which 26 bp are identical to the A+T-rich part of the palindromic sequence which forms the hairpin loop in *ori/rep*.

Conclusions and implications

The functional significance of the sequences we have found in the yeast nuclear genome homologous to mtDNA is probably minimal. Only small portions of the mitochondrial *cob/box* and *var1* genes are represented and these overlap each other in a manner suggesting that they were joined together by deletions of intervening DNA. Further, that portion of the nuclear DNA sequence homologous to mtDNA contains termination signals in all reading frames and includes contiguous sequences corresponding to portions of translated as well as non-translated regions of wild-type mtDNA. Finally, we have been unable to detect any transcripts of this nuclear DNA sequence in ρ^0 yeast strains (F. Farrelly and R.A.B., unpublished observations).

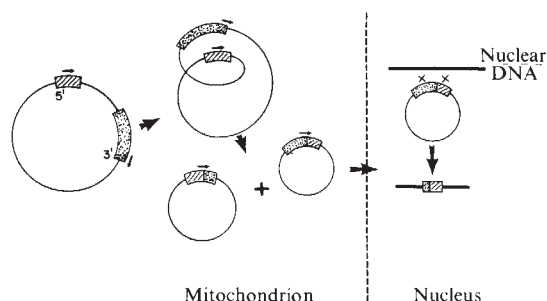


Fig. 6 A model for the transfer and integration of petite mtDNA into the nuclear genome. Homologous regions between *var1* (striped region) and *cob/box* (stippled region) are indicated by the small arrows. Petite mtDNAs can be generated by recombination between these homologous segments.

Possibly, the peculiar sequence we have found is a pseudogene of some formerly expressed nuclear gene. We consider this unlikely, however, because the nuclear sequence contains only small adjoining stretches of DNA from the equivalent of two non-adjacent mitochondrial genes (separated by ~6 kb in the wild-type mitochondrial genome) which encode proteins of seemingly unrelated functions—an electron transport protein (*cob/box*) and a ribosome-associated protein involved in ribosome assembly (*var1*).

Although we cannot formally rule out the possibility that such a sequence arose by some extensive rearrangement of pre-existing nuclear DNA sequences, the arrangement of the sequences homologous to mtDNA suggests they originated from mtDNA, and in particular, from petite mtDNA which may have entered the nucleus and become integrated into chromosomal DNA. A model of this hypothetical event is shown in Fig. 6.

The wild-type yeast mitochondrial genome is known to undergo extensive spontaneous deletions at high frequency (~1%), giving rise to petite (ρ^-) cells; generally, these petites retain only a small part of the wild-type mitochondrial genome in an amplified and often rearranged configuration³⁸. Analysis of mtDNAs from a number of spontaneous petites suggests that these petite DNAs arise as a result of recombination between homologous regions of the wild-type genome³⁹. The consequence of such recombination events is to fuse noncontiguous regions of the wild-type genome such that the regions of homology between these normally separated sequences are retained at the joints. Such mtDNA molecules probably arise frequently even in cells which do not become phenotypically petite.

We propose that the nuclear DNA sequences we have described originated from the integration of such a fragment of petite mtDNA into the nuclear genome (Fig. 6). Sequences from the *cob/box* and *var1* regions might have become joined in a petite mtDNA molecule by recombination between the homologous sequences at these two loci. Additional recombination events could be responsible for the joining of the short G+C-rich sequence from *cob/box* intron 5 to the exon 6 sequence.

The presence of a segment homologous to a portion of a mitochondrial *ori/rep* sequence provides additional evidence that the nuclear sequences were originally derived from mtDNA. In addition to providing some replicative advantage (and therefore stability) for the putative petite mtDNA, it may have allowed for the maintenance of the sequence once integrated into the nuclear genome. Indeed, it has recently been shown that the introduction of mitochondrial *ori/rep* sequences into appropriate yeast plasmids allows the plasmid to replicate

autonomously⁴⁰, indicating that such sequences can function as origins of replication in the yeast nucleus. Thus, once integrated, the maintenance of the mitochondrial sequences would be insured.

If the model presented in Fig. 6 is valid, by what process were these petite mtDNA sequences transported into the nucleus? On the one hand, simple integration of extra-nuclear DNA sequences into chromosomal DNA presents no conceptual difficulty inasmuch as DNA-mediated transformations are commonplace. On the other hand, is there some functional relationship associated with the presence in some yeast strains of Ty elements closely linked to the DNA sequence homologous to mtDNA? One possibility is that the location of such a tandem pair of Ty elements is coincidental and represents only 'stored' copies (ref. 29) of these mobile elements in a non-functional region of the nuclear genome. Alternatively, it is conceivable that the Ty elements have participated directly in the conveyance of these homologous mtDNA sequences to their present location within the nuclear genome. (Their absence, as well as the absence of δ sequences at this location in other yeast strains, could have been the result of excisions by processes other than δ - δ recombinations.)

The transfer and integration of genetic material from extra-nuclear compartments into chromosomal DNA and the possible involvement of multi-copy mobile elements have been suggested for several systems. These pertain to the so-called 'processed' genes⁴¹⁻⁴⁴ which are genomic pseudogene sequences bearing a close resemblance to mRNAs: they lack the intervening sequences of their expressed counterparts and the deletions are precisely at the exon-intron splice junctions, they contain long poly(A) stretches at their 3' ends, and they sometimes lack regulatory and promoter sequences at their 5' ends. These features suggest that processed genes originated by re-integration of reverse transcribed DNA copies of processed mRNAs into the nuclear genome. One conjecture is that mobile genetic elements such as *Alu* (ref. 45), retrovirus sequences⁴⁶ or small nuclear RNAs⁴⁷ can be incorporated into the processed sequence (at the genomic, processed RNA, or cDNA level) to direct and/or facilitate the re-integration of the sequences back into the nuclear genome. Ty elements in yeast could therefore

function in a similar fashion to convey extra-chromosomal genetic material into the nuclear genome.

One additional implication of our findings concerns the evolutionary origin of mitochondria and mitochondrial genomes. The endosymbiotic theory (see ref. 48) suggests that the progenitors of eukaryotic and prokaryotic cells, having evolved from an ancestral cell type, coexisted for some time in evolutionary history. Colonization of the proto-eukaryotic cell then occurred, perhaps quite frequently in an evolutionary time scale, and was followed by selective transfer of genes contained within the invading genome to the host genome. Importantly, genes would have moved from the colonized to the host genome before, as well as after, the nucleus and mitochondria were established as separate organelles. Depending on the prevailing selective conditions at the time of and following invasion, and considering the possibility of multiple colonization events, differential gene transfer leading to the establishment of unique mitochondrial genomes could have taken place. If such transfers are relatively promiscuous, non-functional transfer, as suggested here for the integration of petite mtDNA sequences into the nuclear genome, may also have been relatively frequent. Assuming that the nuclear sequence we have found homologous to mtDNA has not been subjected to selective pressure, we can estimate that such a sequence arose some 25 Myr ago (assuming 7×10^{-9} substitutions per nucleotide site per yr and correcting for multiple events according to Perler *et al.*⁴⁹), a relatively recent event within the time of emergence of modern fungi (~400 Myr). From these considerations we might expect to find other mtDNA sequences, in addition to those described here, in the yeast nuclear genome.

In a recent preliminary report⁵⁰, sequences homologous to mtDNA have been identified in the nuclear genome of *S. purpuratus*. Similar to the results reported here, these sequences apparently represent only portions of mitochondrial gene sequences present in the nuclear genome in a rearranged configuration.

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Positive control and autogenous regulation of the *nifLA* promoter in *Klebsiella pneumoniae*

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The nitrogen fixation (nif) genes of Klebsiella pneumoniae are specifically regulated by the products of the nifLA operon. We have located the promoter of this operon, and identified sequences required for nifLA transcription. Transcription from this promoter is shown to be positively regulated by the ntrC gene product (which coordinates the expression of many operons required for nitrogen assimilation) and also autogenously by the product of the nifA gene.

THE nitrogen fixation gene cluster of *Klebsiella pneumoniae* contains at least 15 *nif* genes, arranged in several operons, which are required for the synthesis and activity of the enzyme nitrogenase^{1,2}. These genes are specifically regulated by two additional genes, *nifL* and *nifA*, which are located in a single operon within the gene cluster. The *nifL* gene product represses *nif* transcription in response to fixed nitrogen (N) and oxygen³⁻⁵ whereas the *nifA* gene product activates *nif* transcription⁶⁻⁸.

The *nif* genes are also subject to regulation by the nitrogen regulatory (*ntr*) genes *ntrA* (*glnF*), *ntrB* (*glnL*) and *ntrC* (*glnG*), which exert a general control on operons subject to nitrogen regulation in enteric bacteria⁹. The *ntrB* and *ntrC* genes are linked to *glnA*, the structural gene for glutamine synthetase, and form a single operon in *Escherichia coli* which is transcribed from *ntrB* to *ntrC*, either from their own promoter or by readthrough transcription from the stronger *glnA* promoter¹⁰⁻¹². Repression of *glnA* transcription is mediated by *ntrB* and *ntrC*^{9,13,14} whereas activation of *glnA* transcription is mediated by *ntrC* in concert with an unlinked regulatory gene, *ntrA*⁹. These four genes have been identified in *K. pneumoniae*¹⁵⁻¹⁸ and it has been shown that *ntrC* (but not *glnA* or *ntrB*) is required for positive activation of *nif* expression¹⁸.

Recent evidence suggests that *ntr* control of *nif* expression is mediated solely at the promoter of the *nifLA* operon^{8,19-21}. The *nifLA* promoter is therefore the primary target for regulation of *nif* transcription in response to the level of fixed N in the growth medium, as regulation at the other *nif* promoters is dependent on the level of *nifLA* transcription. Here we define nucleotide sequences that are important for *nifLA* promoter function, and show that either the *ntrC* or the *nifA* gene products can activate expression from this promoter.

Localization of regulatory sequences upstream of *nifL*

A map of the *nifLA* region is shown in Fig. 1a. To detect sequences determining the initiation of *nifL* transcription and translation, an *EcoRI*-*SmaI* fragment spanning the promoter region was cloned into the translational *lac* fusion vector pMC1403²², which lacks the *lac* regulatory region and the first eight codons at the amino-terminal end of the β -galactosidase (*lacZ*) gene (Fig. 1a). To bring *nifL* into the same reading frame as *lacZ*, a single base pair deletion at the *SmaI* site was selected following digestion and re-ligation of this original clone, yielding plasmid pRD514 (Figs 1b, 2).

Table 1 β -Galactosidase activity of *nif-lac* translational fusions in *K. pneumoniae*

Plasmid	Position of deletion junction(s) on <i>HindIII-SmaI</i> fragment	β -Galactosidase units in:					
		UNF926 (<i>ntr⁺ nifΔ</i>)		UNF931 (<i>ntr⁺ nif⁺</i>)		UNF1829 (<i>ntrC nif⁺</i>)	
		-NH ₄ ⁺	+NH ₄ ⁺	-NH ₄ ⁺	+NH ₄ ⁺	-NH ₄ ⁺	+NH ₄ ⁺
pRD514	—	3,500	100	3,566	90	110	125
pRD526	—	3,320	122	4,220	230	230	238
pRD532	127 (-227)	3,870	160	4,000	146	211	155
pRD531	195 (-159)	935	164	2,244	126	71	98
pJC1	214 to 297	808	121	1,332	161	354	211
pRD535	310 (-44)	735	120	1,115	93	150	111
pRD549	319 (-35)	671	151	803	235	212	243
pRD547	321 (-33)	599	183	1,141	247	144	198
pRD560	326 (-28)	239	60	524	75	73	71
pRD520	327 to 343	70	12	75	4	67	12
pRD559	354 (-1)	57	59	105	54	76	54
pRD529	193 to 370	23	16	29	24	—	—
pRD543	—	568	406	1,851	452	621	518

Overnight cultures (4 ml) of strains were grown anaerobically at 28 °C in bijoux bottles in nitrogen-free medium (NFD) containing 200 μ g ml⁻¹ carbenicillin, 50 μ g ml⁻¹ histidine, 100 μ g ml⁻¹ serine (-NH₄⁺) or with the addition of 2 mg ml⁻¹ (NH₄)₂SO₄ (+NH₄⁺). β -galactosidase activities were measured as described in ref. 6. Relevant genotypes are: UNF926 (Δ (*his-nif*) 2632 Δ *lac2001 recA hsdRI*), UNF931 (*hisD2* Δ *lac2002 recA hsdRI*) and UNF1829 (*ntrC36::Tn7 hisD2* Δ *lac2002 recA hsdRI*).

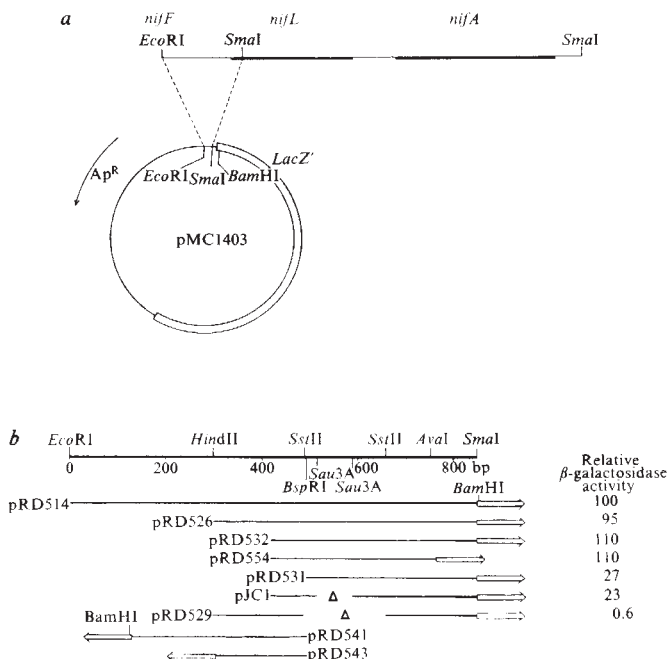


Fig. 1 *a*, Map of the *nifLA* region of the *K. pneumoniae* genome^{25,43} showing the *EcoRI-SmaI* fragment cloned into the translational *lac* fusion vector pMC1403³². *b*, Enlarged map of the *nif EcoRI-SmaI* fragment. Only those restriction sites used for plasmid construction or determination of the start site for *nifLA* transcription (Fig. 3) are shown. Lines beneath the map denote *nif* fragments cloned into pMC1403 and the wide arrows indicate *lacZ'* DNA and the direction of its transcription relative to the inserted fragment. All the plasmids shown carry a *BamHI* site adjacent to the *nif-lac* junction at one end of the inserted fragment and an *EcoRI* site flanking the other end. Plasmid pRD514 lacks a single nucleotide at the *SmaI* site in *nifL*, bringing the translational reading frame of *nifL* into concordance with that of *lacZ'* (Fig. 2). The relative β -galactosidase activities refer to the level of activity given by each plasmid in UNF926 grown in the absence of ammonia (Table 1).

Methods: The *EcoRI-BamHI* fragment from pRD514 was purified by electroelution from an agarose gel⁴⁴, cut with *HindII* and ligated to a *SmaI-BamHI* digest of pMC1403, forming pRD526. Plasmid pRD532 was derived following nuclease *Bal31* digestion from the *EcoRI* site in pRD514; shortened *nif* fragments were excised with *BamHI* and re-cloned into *SmaI-BamHI* digested pMC1403. The *EcoRI-BamHI* fragment from pRD532 was further digested with *Sau3A* and re-cloned into (*EcoRI-BamHI* digested pMC1403 to yield pJC1. The deletion on pRD529 was derived in a similar fashion following *SstII* digestion of the *EcoRI-BamHI* fragment from pRD526. The structure of both of these deletions was confirmed by DNA sequencing. pRD554 was obtained by nuclease *Bal31* digestion from the *BamHI* site of pRD532, followed by excision of the shortened *nif* DNA with *EcoRI*. The excised fragments were ligated to an *EcoRI-SmaI* digest of pMC1403, transformed into *E. coli*, and colonies expressing β -galactosidase on X-gal plates^{6,45} were picked. pRD531 was obtained following digestion of the *EcoRI-BamHI* fragment from pRD526 with *BspRI*. Plasmids pRD541 and pRD543 were derived from pRD516 (not shown) which contains three contiguous *BspRI* fragments cloned into the *SmaI* site of pMC1403. The *nif* DNA on pRD516 extends from the *BspRI* site shown on the map to another *BspRI* site located 20 bp to the right of the *EcoRI* site, and is inserted in the opposite orientation in pMC1403 to the *nif* DNA on plasmid pRD514. pRD516 was digested with *BamHI*, treated with nuclease *Bal31*; the resected *nif* fragments were excised with *EcoRI* and re-cloned into *EcoRI, SmaI* digested pMC1403. pRD541 and pRD543 are two of the in-frame translational fusions resulting from this procedure.

A second in-frame *lac*-fusion was derived from plasmid pRD514 by deleting sequences upstream from the *SmaI* site with exonuclease *Bal31*. Both of these plasmids gave similar levels of β -galactosidase activity. Sequencing their fusion junctions established the reading frame of *nifL*, as the frame of *lacZ'* relative to the *SmaI* site on pMC1403 is known. Only the first of the three in-frame ATG codons occurring at positions 427, 439 and 442 in the *HindII-SmaI* fragment is immediately preceded by a Shine and Dalgarno sequence AGGAG (see Fig. 2). It is followed by an open reading frame and so is probably the initiation codon of *nifL*.

Deletions were constructed to localize promoter sequences on pRD514 (Fig. 1*b*). Plasmids pRD526 and pRD532, which carry the entire intergenic region preceding *nifL*, gave high levels of β -galactosidase activity in a *nif* deletion background. This activity was repressed more than 20-fold when strains were grown on ammonia, as expected for genes under *ntr* control. The deletion of sequences downstream of the left *SstII* site (for example pJC1) resulted in a decrease in β -galactosidase activity (Fig. 1*b*). Deletion of the internal *SstII* fragment (pRD529) eliminated promoter activity, indicating that sequences required for transcription of the *nifLA* operon are located between these two *SstII* sites.

When DNA fragments from the region of interest were cloned in the reverse orientation in pMC1403, we found that β -galactosidase activity was also obtained. Plasmids with in-frame *nif-lac* fusions, pRD541 and pRD543, were derived from pRD516 using *Bal31* as described in Fig. 1*b* legend. The reading frame obtained from the sequence of the fusion junction of pRD543 is open downstream from the ATG codon at position 105, which is immediately preceded by a Shine and Dalgarno sequence AGGAG (see Fig. 2). We assume that the promoter for this gene is located between this Shine and Dalgarno sequence and the *BspRI* site shown in Fig. 2. The β -galactosidase activity determined by plasmid pRD543 is not affected by ammonia or by an *ntrC* mutation, indicating that this promoter is constitutively active (Table 1). However, the level of activity was increased in a *nif*⁺ background in the absence of ammonia, suggesting that expression can be enhanced by a *nif* product in *trans*. Studies with a transcriptional *nif-lac* fusion suggest that *nifF* is transcribed in the same direction as *nifLA*²⁴, although the transcripts and polypeptide products determined by this region have not been unambiguously identified²⁵ and there is a suggestion that two polypeptides of molecular weights 20,000 and 26,000 are encoded by this region⁷. The unidentified promoter and associated coding sequence may determine synthesis of one of these polypeptides, possibly the *nifF* gene product itself.

Determination of the start site for *nifLA* transcription

The transcriptional start point was determined by generating a labelled DNA fragment extending from the *BamHI* site shown in Fig. 2 to the 5' terminus of the *nifL* mRNA and electrophoresing this adjacent to a sequencing ladder obtained using dideoxy reactions with a primer originating at the same *BamHI* site. Two different methods were used to generate this fragment. The first used *S*₁ nuclease to degrade hybrids formed between mRNA and a 350-base pair (bp) *EcoRI-BamHI* fragment cut from pRD531; the second used reverse transcriptase to extend a 98-bp *AvaI-BamHI* fragment hybridized to mRNA. In both cases total mRNA from derepressed cells carrying pRD526 was used. Each method is susceptible to different artefacts. The *S*₁ digestion products showed minor length heterogeneity (Fig. 3*a*, lane 3), and the reverse transcriptase products (Fig. 3*b*) included somewhat shorter fragments corresponding to prematurely terminated cDNA copies. Comparing these results, however, allows the unambiguous identification of position 354 on the *HindII-SmaI* fragment as the start of transcription. Fragments corresponding to this position were not observed when RNA from ammonia-repressed cells was used.

Table 2 Activation of *nifL* expression by *ntnC* and *nifA* plasmids

<i>nifL</i> plasmid	Deletion junction(s)	Plasmids in trans					
		None		pMM14 (<i>ntnC</i> ⁺)		pMC71A (<i>nifA</i> ⁺)	
		-NH ₄ ⁺	+NH ₄ ⁺	-NH ₄ ⁺	+NH ₄ ⁺	-NH ₄ ⁺	+NH ₄ ⁺
pRD526	—	127	116	5,320	6,250	4,500	6,050
pRD532	-227	89	77	3,380	4,580	3,680	3,240
pRD531	-159	73	22	2,860	1,450	4,080	5,225
pJC1	-140 to -57	134	147	636	321	1,072	2,057
pRD535	-44	113	74	829	518	1,870	1,600
pRD549	-35	125	121	431	336	964	1,036
pRD547	-33	144	98	425	260	896	801
pRD560	-28	77	40	237	139	761	632
pRD520	-27 to -12	35	9	32	8	13	7
pRD559	-1	80	38	71	50	50	53
pRD529	-161 to +17	17	15	21	21	21	22

Cultures (4 ml) were grown anaerobically in NFD medium containing 200 $\mu\text{g ml}^{-1}$ glutamine and 100 $\mu\text{g ml}^{-1}$ carbenicillin either in the absence (-NH₄⁺) or presence (+NH₄⁺) of 2 mg ml⁻¹ (NH₄)₂SO₄. Cultures containing pMM14 or pMC71A were selected with 30 $\mu\text{g ml}^{-1}$ kanamycin and 15 $\mu\text{g ml}^{-1}$ chloramphenicol respectively. β -galactosidase activities were measured as described previously⁶. The strain background in all cases was *Escherichia coli* ET8894 ($\Delta(rha-ntnC)1703$ *rbs gyrA hutC lacZ* :: ISI Mucts 62)⁴².

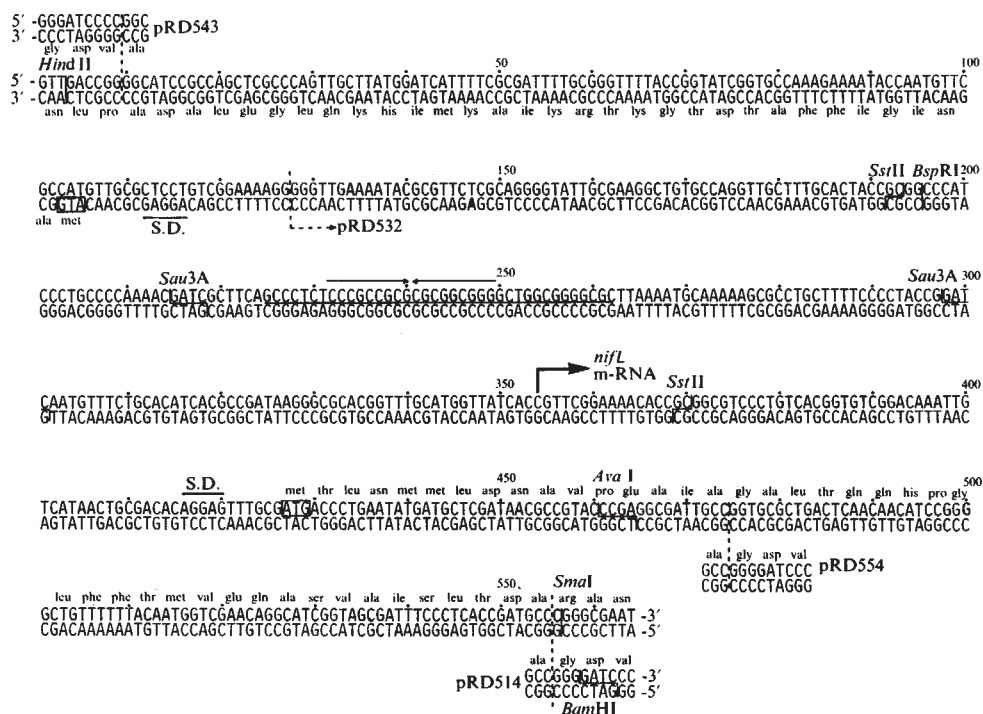
Deletions affecting *nifLA* promoter function

By introducing plasmids into various mutant backgrounds and measuring β -galactosidase activity we have carried out a functional analysis of the *nifLA* promoter. Previous studies have suggested that the *nifA* gene product is not required for *nifLA* transcription. In accordance with these earlier observations expression from the complete promoter (carried on plasmids pRD514, pRD526 and pRD532) was unaffected by the presence of a chromosomal copy of the *nifA* gene in trans (Table 1). The gene dosage effect on *lacZ* expression from these multi-copy plasmids suggests that transcription from the *nifLA* promoter is not limited by the level of transcriptional activators. Similar results have also been found by direct transcription measurements with another multi-copy plasmid carrying the

nifLA promoter⁵. Table 1 also shows that a *ntnC* mutation reduces expression from the *nifLA* promoter by at least an order of magnitude in plasmids containing the entire intergenic region, implying that the *ntnC* gene product is required for *nifLA* expression. The level of activity in strains carrying a *ntnC* mutation is approximately equivalent to the level of activity obtained with *ntnC*⁺ strains grown in the presence of ammonia.

We have constructed a number of deletions in the intergenic region *in vitro*. The DNA sequence spanning the deletion junctions has been determined as shown in Figs 2 and 4, and the β -galactosidase activity of each deletion has been determined (Table 1). Surprisingly, deletion of nucleotides upstream of the *Bsp*RI site (plasmid pRD531), which is 150 bp from the transcriptional start site, reduced the promoter activity fourfold in a *nif* deletion background. The level of expression progressively

Fig. 2 Nucleotide sequence of the *Hind*II-*Sma*I fragment, which spans the intergenic region between *nifF* and *nifL*. The *nif-lac* junctions of plasmids pRD514, pRD554 and pRD543 are indicated by dotted lines and the vector sequence at the junction (which contains a *Bam*HI site immediately adjacent to the eighth codon of *lacZ*²²) is shown above or below the *nif* sequence. The right-hand end of the *nif* DNA on plasmid pRD532 (see Fig. 1) is also indicated by a dotted line. The line between the DNA strands from position 225 to 262 denotes the G+C-rich region discussed in the text; thin arrows indicate a palindrome within this sequence. The thick arrow shows the start-point for *nifLA* transcription. Putative AUG initiation codons are boxed and possible Shine-Dalgarno²³ (S.D.) sequences are marked by horizontal bars. Amino acid sequences predicted from the DNA sequence are also shown. The DNA sequence was determined by the dideoxy technique^{46,47} using inserts cloned into M13mp7, M13mp8 and M13mp9 vectors^{48,49}, and a 17-bp synthetic primer (M. Gait, unpublished). Both strands were sequenced using a variety of overlapping clones and the data were compiled by computer analysis⁵⁰.



decreased to 7% of the full promoter activity in a deletion extending to position 326, which corresponds to nucleotide -28 (pRD560, Table 1). The residual activity in these deletions was still subject to ammonia repression and was dependent on *ntrC*, indicating that the promoter remained subject to *ntr* control. The derepressed level of activity was enhanced when these plasmids were introduced into a *nif*⁺ background (Table 1), suggesting that a *nif* gene product can stimulate expression from these deletions in *trans*, unlike plasmids which carry the entire intergenic region. Deletions removing the -10 region of the promoter (for example, pRD559) eliminated promoter activity. Promoter function was also abolished in a deletion extending from positions 327 to 343 (-26 to -12; pRD520, Table 1).

Activation of the *nifLA* promoter by the *ntrC* and *nifA* gene products

The above results predict that *ntrC* is a positive activator of *nifLA* transcription. In addition, the *trans*-activation of deletions in a *nif*⁺ background (Table 1) indicates that a *nif*-specific regulatory molecule may activate *nifLA* expression. We have now shown that the *nifA* gene product will activate transcription at its own promoter, an effect normally masked by *ntr* mediated control.

To examine the effects of the *ntrC* and *nifA* gene products on *nifLA* expression we have introduced plasmids that produce either of these gene products constitutively from vector promoters in *trans* to a *nifL-lac* fusion plasmid. The plasmids used were pMM14, in which transcription is dependent on the kanamycin promoter on pACYC177, and pMC71A⁸, which expresses *nifA* from the promoter of the tetracycline resistance gene on pACYC184. pMM14 is a derivative of pMM12¹⁸, which carries a Tn5 insertion in the ampicillin resistance gene. An *E. coli* strain carrying a *glnA ntrBC* deletion was used for these experiments. When introduced into this strain none of the *nifL-lac* fusion plasmids alone gave activated levels of *lac* expression (Table 2), because nitrogen regulatory control is absent in this strain. *nifL-lac* fusions carrying the entire inter-

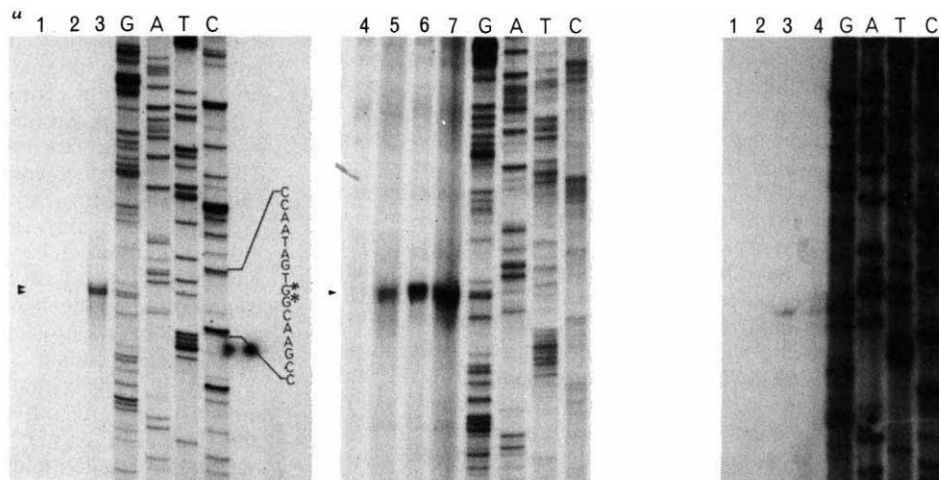
genic region showed constitutive levels of β -galactosidase activity when either the *ntrC* or *nifA* plasmid was present in *trans*. Control experiments with plasmid pRD557, which carries a *KpnI* deletion removing the carboxy-terminal end of *ntrC*, or with plasmid pACYC184 (the vector used for construction of pMC71A), showed no activation of *nifL* expression (data not shown). Thus both *ntrC* and *nifA* can activate expression from the *nifLA* promoter. These results also show that *glnA* and *ntrB* are not required for *nifL* expression, confirming previous findings with nitrogenase activity measurements¹⁸.

The various promoter deletions were also tested for activation by the *ntrC* and *nifA* plasmids. Stepwise deletion of the sequence from nucleotide -157 to -28 progressively decreased the ability of the *ntrC* plasmid to activate *nifL* expression (Table 2). The *nifA* plasmid was apparently more efficient at activating expression from these deletions, but again a progressive decrease in the level of activation was observed. In general these results parallel those obtained with the *ntr*⁺ *nif* deletion (UNF926) and *ntr*⁺ *nif*⁺ (UNF931) backgrounds in Table 1, suggesting that the enhancement of activation in a *nif*⁺ strain is due to the presence of the *nifA* gene product. No activation was observed with pRD559, which carries a deletion extending through the transcriptional start site, nor with pRD520, which lacks nucleotides between -27 and -12.

The finding that activation by *ntrC* and *nifA* plasmids was not completely abolished by deletions extending into the -35 region of the promoter was surprising as this region is important for recognition by RNA polymerase in many prokaryotic promoters^{26,27}. It was therefore important to check that activation of *nifL* expression by *ntrC* and *nifA* plasmids was mediated at the transcriptional level, and that the resultant transcripts were initiated at the same site in the *nifLA* promoter as in the wild-type (*ntrC*⁺) strain. Figure 3 shows that in strains carrying either *ntrC* or *nifA* plasmids, transcription was indeed initiated at the same position to that found in *ntr*⁺ cultures. The *nifL* transcript was not detected in strains lacking the activating plasmid, indicating that activation by *ntrC* and *nifA* occurs at the transcriptional level.

Fig. 3 Identification of the start point for *nifLA* transcription. *a*, S₁ nuclease mapping. The protected fragments described below (see methods) are shown alongside the dideoxy sequencing fragments in lanes G, A, T and C. The RNAs used in the hybridizations were obtained from the following sources: lane 1, yeast tRNA (control); lane 2, UNF926 (pRD526) +NH₄⁺; lanes 3 and 5, UNF926 (pRD526) -NH₄⁺; lane 4, ET8894 (pRD526) -NH₄⁺; lane 6, ET8894 (pRD526) (pMM14; *ntrC*⁺) -NH₄⁺; lane 7, ET8894 (pRD526) (pMC71A; *nifA*⁺). *b*, Reverse transcriptase mapping using RNA extracted from cultures of the following: lane 1, ET8894 (pRD526) -NH₄⁺; lane 2, UNF926 (pRD526) +NH₄⁺; lane 3, UNF926 (pRD526) -NH₄⁺; lane 4, ET8894 (pRD526) (pMM14; *ntrC*⁺) -NH₄⁺. Strains are described in Tables 1 and 2.

Methods: *a*, To prepare the probe for S₁ mapping pRD531 DNA was digested with *EcoRI*, resected with exonuclease III and filled in with the Klenow fragment of DNA polymerase I using [α -³²P]dATP (400 Ci mmol⁻¹, Amersham). The labelled DNA was re-digested with *BamHI*, and the 370-bp *EcoRI-BamHI* fragment (Figs 1, 2) was purified by electrophoresis on 5% polyacrylamide gels. The probe was mixed with 50 μ g of RNA, lyophilized and redissolved in 30 μ l of hybridization buffer (20 mM HEPES pH 6.5, 0.4 M NaCl, 80% formamide), heated at 80 °C for 10 min, incubated at 50 °C for 3 h, then allowed to cool slowly to 43 °C. After ethanol precipitation the nucleic acids were resuspended in S₁ buffer (30 mM sodium acetate pH 4.5, 3 mM ZnCl₂, 300 mM NaCl) containing 20 μ g ml⁻¹ denatured herring sperm DNA and were treated with 2,000 U of S₁ nuclease (Boehringer) for 1 h at 28 °C. The digestion products were analysed on 6% polyacrylamide gels and sized by comparison with fragments produced in dideoxy sequencing reactions using unlabelled *AvaI-BamHI* fragment (see Fig. 2) as primer (lanes marked G, A, T, C). *b*, The probe for the reverse transcription mapping was a 97-bp *AvaI-BamHI* fragment (Fig. 2), labelled by resection and fill-in with DNA polymerase as described in *a*. The labelled probe was hybridized with RNA prepared from a variety of cultures as in *a*, and incubated with 75 U of reverse transcriptase (Boehringer) in the presence of 0.1 mM of each nucleotide triphosphate, as described elsewhere^{51,52}. The products were analysed on 6% polyacrylamide gels and were run in parallel with dideoxy sequencing reactions (lanes marked G, A, T, C) using the *EcoRI-BamHI* fragment from pRD532 sub-cloned in M13mp8 as template and unlabelled *AvaI-BamHI* fragment as primer.



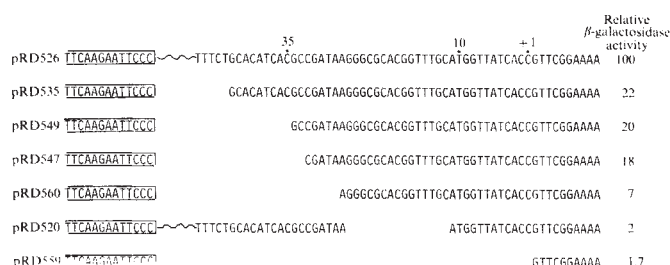


Fig. 4 Nucleotide sequence of deletions in the vicinity of the *nifL* transcriptional start site. The boxed nucleotides are vector sequences and the gaps indicate the extent of the deletion. The wavy lines indicate that the upstream *nif* sequence is intact. Most of these deletions were derived either from pRD514 or from pRD532 using the procedure described for the construction of pRD532 in Fig. 1. The construction of pRD520 will be described in detail elsewhere. Relative β -galactosidase activities refer to the level of activity given by each plasmid in UNF926 grown in the absence of ammonia (see Table 1).

Conclusions

The nucleotide sequence of the *K. pneumoniae nifLA* promoter is atypical among prokaryotic promoters, as might be expected for an operon that is subject to positive control. Inspection of the -10 region reveals a likely Pribnow box²⁸, CATGGT. The sequence 35 bp upstream from the transcription start shows no correlation with the -35 consensus sequence^{26,27}. It is surprising that both promoter function and positive control are retained to some extent in deletions extending as far as nucleotide -28 . These results contrast with the effects of upstream deletions in other positively controlled systems such as *lac*²⁹ and *araBAD*³⁰, where deletions in the -60 region eliminate positive control by disrupting the activator binding site. The retention of some positive control in deletions removing the -35 region of the *nifLA* promoter would tend to preclude binding of activator molecules to an upstream site as the sole mechanism of promoting transcriptional activation at this promoter. Promoter activation could involve direct contact between the activator and RNA polymerase, as has been proposed for the *cat*³¹ and λP_{RM} promoters³².

Another notable feature of the *nifLA* promoter is the apparent requirement for sequences 150 bp upstream from the transcriptional initiation site for maximum promoter activity. This effect is most noticeable in strains lacking the *nifA* gene product. We have detected a second promoter in this upstream region from which transcription is initiated in the opposite direction to that of *nifLA*. Deletions in this second promoter might have an effect on transcription from the *nifLA* promoter due either to the proximity of these promoters to each other, or to some common feature of their regulation. Examination of the DNA sequence between the two promoters reveals a perfect 9-bp palindrome composed entirely of G·C base pairs within an extremely G+C-rich region located between positions 225 and 262 in Fig. 2. A deletion encompassing this region (pJC1) results in a 77% decrease in promoter activity and thus this palindrome might have a significant role in promoter function. G+C-rich sequences have also been observed in the regulatory regions of the *malB*³³ and *malPQ*³⁴ operons of *E. coli*, and it has been suggested that such sequences have a role in positive activation mediated by the *malT* product.

The *ntrC* and *nifA* gene products have many common features. They are both basic polypeptides with very similar molecular weights and isoelectric points, and both require *ntrA* gene function for their regulatory activity³⁵⁻³⁷. Furthermore, the *nifA* product can substitute for the *ntrC* product in transcriptional activation of a number of genes, including *hutUH*, genes for utilization of arginine and proline^{35,36}, and *nifLA* (this paper). The *ntrC* product cannot, however, activate *K. pneumoniae nifH* transcription^{35,36}.

In view of these similarities we might expect that the mode of activation mediated by these two gene products would be analogous, and that promoters of genes under *ntrC* or *nifA*

control might show some sequence homology. Examination of the promoter sequences for the *K. pneumoniae* (refs 38, 39 and M. Cannon and F. Cannon, unpublished data) and *Rhizobium meliloti*^{39,40} *nifH* genes reveals a region of homology with the *K. pneumoniae nifL* promoter between nucleotides -11 and -22 .

The sequences are as follows (regions of homology underlined):

K. pneumoniae nifL

-40 -30 -20 -10 $+1$
ACATCAGCGCGATAAGGGCGCACGGTTGCATGTTATCACCC

K. pneumoniae nifH

-40 -30 -20 -10 $+1$
ATAAACAGGCACGGCTGGTATGTTCCCTGCACCTTCTCTGCTG

R. meliloti nifH

-40 -30 -20 -10 $+1$
TTATTTTCAGACGGCTGGCAGCACTTTTGACCATCAGCCCTG

Sundaresan *et al.* (ref. 39 and unpublished results) have shown that the *R. meliloti nifH* promoter, unlike the *K. pneumoniae nifH* promoter, is subject to both *ntrC* and *nifA* control. Although both the *K. pneumoniae* and *R. meliloti nifH* promoters share homology in the -30 region with the *K. pneumoniae nifL* promoter, the *K. pneumoniae nifH* sequence is less homologous with the other two promoters in the -20 to -10 region, which may reflect the absence of *ntrC* mediated control at this promoter. We also find similar homologies in sequences upstream of the *E. coli glnA* gene (kindly provided by A. Covarrubias), and the *Salmonella typhimurium dhuA* and *argTr* genes⁴¹, which are also subject to nitrogen control, but the precise start points for transcription of these genes have not yet been determined. Higgins and Ames⁴¹ have located sequences with mirror symmetry in the regulatory regions of both the *argTr* and *dhuA* genes, and have postulated that these sequences may be involved in nitrogen control. We do not find extensive mirror symmetries in the *nifL* promoter, so it is unlikely that such sequences are involved in *ntr* control of this promoter.

Regulation of *nif* transcription is mediated at two different levels. First, the *ntr* gene system ensures that *nifLA* transcription is regulated according to the level of fixed nitrogen in the growth medium. Second, the *nifL* and *nifA* gene products specifically modulate transcription of the other *nif* operons. The synthesis of a specific activator and repressor for *nif* transcription allows nitrogenase synthesis to be regulated in response to oxygen and low levels of fixed nitrogen independently of other systems under *ntr* control. What is the physiological significance of autogenous regulation of *nifLA* transcription by the *nifA* gene product? It is doubtful that this activation has a major role in *nif* regulation, as in steady-state conditions we cannot observe autogenous regulation of this operon. It is likely that autogenous regulation by *nifA* (and possibly also by *nifL*) is normally overridden by the *ntrBC* genes and hence is only observed in their absence. However autogenous regulation might play a transient part in situations where the *ntr* gene products become limiting, for example during a marked N-source shift-down.

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Regulation of nitrogen metabolism genes by *nifA* gene product in *Klebsiella pneumoniae*

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The Klebsiella pneumoniae nifA gene product, which is known to activate expression of the nitrogen fixation (nif) structural genes, is shown here also to be able to substitute for the product of the gene glnG (ntrC) in the regulation of other nitrogen metabolism genes. An evolutionary relationship between the nifA and glnG genes is suggested.

BIOLOGICAL nitrogen fixation is the enzymatic reduction of atmospheric dinitrogen (N_2) to ammonia (NH_4^+). The free-living nitrogen fixing species *Klebsiella pneumoniae* contains a cluster of at least 17 contiguous nitrogen fixation (*nif*) genes, arranged in 7 or 8 transcription units (for reviews, see refs 1, 2). Two genes, *nifL* and *nifA*, which comprise a single operon, have regulatory roles. The expression of the *nifLA* operon occurs only at low levels of NH_4^+ , and the *nifA* gene product is required for activation of all *nif* operons except its own^{3–6}. The *nifL* gene product mediates oxygen repression, and, to a lesser extent, NH_4^+ repression^{6–8}.

In several enteric species, transcriptional regulation of genes involved in nitrogen assimilation is centrally controlled (for reviews, see refs 9, 10). Recent studies have shown that the positive transcriptional regulators of several genes involved in nitrogen assimilation are the products of the genes *glnG* (*ntrC*)^{11–16} and *glnF* (*ntrA*)^{12,17,18}, while negative regulation involves the *glnG* product^{19,20}, and in addition, the products of genes *glnL* (*ntrB*)^{19,21,22} and *glnB* (refs 23, 24 and R. Bueno, G. Pahel and B. Magasanik, in preparation). Previous studies of *nif* regulation suggested that the *nifLA* operon is also under the control of the centralized nitrogen control system, and regulated analogously to the genes governing the utilization of histidine (*hut*), proline (*put*), arginine (*aut*) and the biosynthesis of glutamine (*glnALG*)^{14,15}. Figure 1 shows the overall structure of the *nif* gene cluster and a summary of *nif* control pathways as currently perceived.

In this article, we report two novel discoveries: (1) the *nifA* gene product can substitute for the *glnG* gene product in its regulatory roles, including activation of the *nifLA* operon; and

(2) gene activation by the *nifA* product requires the *glnF* product, with the possible exception of the *nifLA* promoter. Our data are in agreement with previous suggestions that the *glnG* product regulates expression of the *nifLA* operon^{14,15}. In addition, our data demonstrate that the *glnF*, *glnL*, and *glnB* gene products, directly or indirectly, also regulate expression of the *nifLA* operon. Because of the similarities between the *glnG* and *nifA* genes in terms of function, size and structural arrangements in their respective operons, we propose that the *nifLA* operon evolved directly from an ancestral *glnLG* transcription unit.

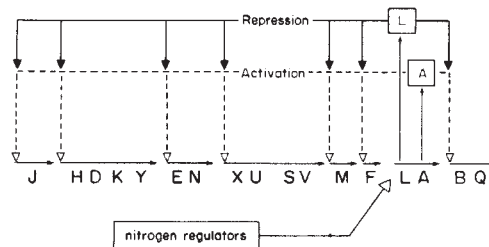


Fig. 1 Annotated genetic map of the *K. pneumoniae nif* cluster. The 17 genes in the *nif* cluster (*nifQ*–*nifJ*) are divided into eight transcription units as indicated. The *nifA* and *nifL* products regulate the expression of the other *nif* operons. The *nifK* and *nifD* products are the α and β subunits of the nitrogenase MoFe protein and the *nifH* product is the subunit of the nitrogenase Fe protein. The *nifM* and *nifS* products have been implicated in the processing of *nifH* product and the *nifV* product is necessary for FeMo co-factor maturation. The *nifF* and *nifJ* products transport electrons from pyruvate to nitrogenase. The *nifN*, *nifE* and *nifB* products are necessary for FeMo co-factor synthesis and/or processing. The roles of the *nifQ*, *nifU*, *nifX* and *nifY* products are not known.

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Table 1 Description and sources of bacterial strains used

Strain	Description	Source or reference
<i>E. coli</i> K12		
FDB213	<i>endA1, thi-1, hsdR17, supE44, gln(ΔLG)Δ 2000, recA56, srl</i> (isogenic with YMC9)	F. de Bruijn
YMC9	<i>endA1, thi-1, hsdR17, supE44, lacΔ U169</i>	21
YMC17	YMC9 <i>glnG::Tn5</i>	21
TH1	YMC9 <i>glnFΔ</i>	T. Hunt
D01413	YMC9 <i>glnDΔ</i>	This work*
YMC10	YMC9 <i>hutC_{K.aerogenes}</i>	21
YMC11	YMC10 <i>gln(ΔLG)Δ 2000</i>	21
YMC15	YMC10 <i>glnL302</i>	22
NK6986	<i>Δlacpro_{XIII}, recA, thi, supE, rpsL</i>	N. Kleckner
NK7047	<i>Δlacpro_{XIII}, thi, val^R, rpsE/F^R lac^{O1} lacZ::Tn5pro⁺</i>	N. Kleckner
ET6362	<i>araD, thi, rpsL, rhaD, lacΔ U169, glnF::Tn10, glnG::Tn5</i>	21
<i>K. pneumoniae</i> M5a1		
KP5614	<i>hisD2, hsdR1, recA56, srl</i>	47
KP5060	<i>hisD2, hsdR1, glnA100(GlnA⁻GlnG⁻)</i>	48
<i>K. aerogenes</i>		
CG566	<i>metB4, rha-1, nadB1</i>	35
DO1490	CG566 <i>glnG229::Tn5</i>	This work*
Plasmids and phages		
pGR378	Ap ^r <i>nifL</i> promoter, pBR322 derivative	33
pWB6	Ap ^r Tc ^s , pBR322 derivative	W. Buikema
pGR398	Ap ^r <i>nifA</i> , pACYC177 derivative	33†
pGR397	Ap ^r , Km promoter- <i>nifA</i> ⁺ , pACYC177 derivative	33†
pFB54	Cm ^r <i>glnA</i> ⁺ , pACYC184 derivative	14
pFB514	Cm ^r <i>glnA</i> ⁺ G ⁺ , pACYC184 derivative	14
pglN53	Ap ^r Tc ^s <i>glnA</i> promoter- <i>glnG</i> fusion, pBR322 derivative	22
pglN53Y	Ap ^r Tc ^s derivative of pglN53	This work*
pDO503	Tc ^r <i>nifL</i> ⁺ A ⁺ B ⁺ Q ⁺ , pRK288 derivative	This work*
pDO504	Tc ^r <i>nifLΔ</i> A ⁺ B ⁺ Q ⁺ , pRK248 derivative	This work*
pDO531	Tc ^r <i>nifL-lacZ</i> fusion, from pDO504	This work*
pDO516	Ap ^r <i>lacUV5</i> promoter- <i>nifA</i> ⁺ fusion, pBR322 derivative	This work*
pVSA3	Km ^r <i>nifH-lacZ</i> fusion, cloned in P4DO105 (a phage P4 phasmid vector)	V. Sundaresan‡
λgln101	<i>glnA</i> promoter- <i>lacZ</i> fusion, cloned in λ132	21

* DO1413 is a Tc^s derivative of YMC9 *glnD::Tn10* obtained through ampicillin enrichment. DO1490 is made by P1 transduction using a lysate (a gift of L. Reitzer) prepared from strain LR86 (ref. 35). pglN53Y is a Tc^s derivative of pglN53 constructed by destroying the *Bam*HI restriction site in the Tc^r gene. pglN53 was opened with *Bam*HI and recircularized by blunt end ligation after first filling the *Bam*HI staggered ends with dXTPs and DNA polymerase I (large fragment). The structures and constructions of pDO503, pDO504, pDO531 and pDO516 are described in Fig. 2 legend.

† pGR398 and pGR397 both carry the *nifA*⁺ gene on a *Xma*I restriction fragment inserted into the *Xma*I site of pACYC177, but differ in the orientation of their inserts. In pGR397, *nifA* is transcriptionally fused to the constitutive kanamycin promoter of pACYC177.

‡ pVSA3 consists of a *nifH-lacZ* protein fusion²⁹ inserted into P4DO105, a phage P4 phasmid vector²⁸. P4DO105 can only replicate as a plasmid in a Su⁺ host but can integrate by P4 site-specific recombination into a Su⁻ host (D.O. and F.A., manuscript in preparation).

Localization of the *nifLA* promoter

Figure 2 shows the overall structure of the *nifLA* operon. The approximate location of the *nifLA* promoter was determined by deletion analysis using exonuclease *Bal*31 to generate a set of deletions in plasmid pDO503 (see Table 1), starting at the *Bgl*II site. The set of deleted plasmids was used *in vivo* in complementation studies in a *nifA*⁻ host to identify plasmids that failed to complement due to deletion of the 5' end of the *nifL* gene. The precise mRNA start site was determined by S₁ nuclease mapping²⁵ using purified RNA synthesized *in vivo*. The *nifL* protein start codon was identified by determining the DNA sequence upstream from the *Bgl*II site for approximately 400 bp (data not shown) and then aligning the DNA sequence with the N-terminal amino acid sequence of the *nifL* protein, which was obtained in collaboration with M. Chance and W. H. Orme-Johnson (data not shown). A detailed description of the *nifLA* operon, including the data to support the features shown in Fig. 2, will be published separately.

One of the plasmids used in the deletion analysis of the *nifLA* operon was pDO504, which consists of an *Eco*RI fragment encoding *nifLΔ A⁺B⁺Q⁺* cloned in pRK248 (ref. 26), a low copy plasmid vector derived from RK2. During the construction of pDO504, the *nifL*-internal deletion (generated *in vitro* with exonuclease *Bal*31) was recircularized with *Bam*HI molecular linkers. Insertion of the *Bam*HI-*Sal*I *lacZY* fragment from pMC1403 (ref. 27) into pDO504 yielded a *nifL-lacZ* in-frame protein fusion (pDO531). The *nifL-lacZ* fusion in pDO531 allowed us to readily monitor the expression of the *nifLA* promoter by monitoring β-galactosidase activity. Details of the structures and constructions of pDO504 and pDO531 are described in Fig. 2 legend.

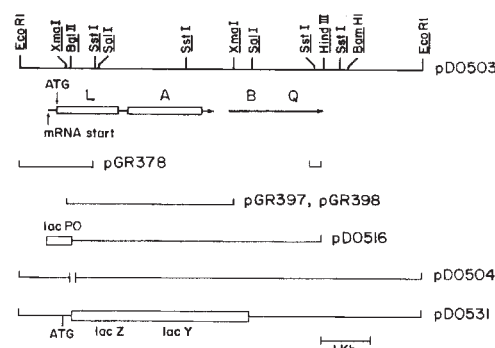


Fig. 2 Physical and genetic map of the *nifLABQ* region of *K. pneumoniae* and its plasmid derivatives. *lacPO_{UV5}* and *lacZY* are not drawn to scale. *lacPO* is about 0.1 kilobases (kb) and *lacZY* is about 6.2 kb. Endonuclease restriction sites relevant to the text are from Riedel *et al.*³³ and our unpublished results. The construction of pGR397, pGR398 and pGR378 has been described previously³³. pDO516 is derived from pGR118³³ by substituting the *Eco*RI-*Bgl*II *nifL* promoter fragment with an *Eco*RI-*Bam*HI *lacPO_{UV5}* fragment. pDO504 is the 8.0-kb *Eco*RI fragment inserted into the 9.6-kb low copy plasmid pRK248²⁶ in the orientation of the Tc^r gene proximal to *nifL*. The following modifications are present on pDO504: (1) The *Bgl*II site on pRK248 and the *Bam*HI site distal to *nifBQ* were eliminated by treating the endonuclease-cleaved staggered ends with dXTPs and DNA polymerase I (large fragment), followed by blunt end ligation. (2) A small deletion within the *nifL* gene was generated by opening the remaining *Bgl*II site with endonuclease, treating the ends with limited *Bal*31 exonuclease digestion, and recircularizing the plasmid with *Bam*HI molecular linkers. A segment of approximately 240 base pairs internal to *nifL* is deleted in pDO504 in pDO504. The *Bam*HI-*Sal*I *lacZY* fragment of pMC1403²⁷ was inserted into pDO504 by a *Bam*HI-*Sal*I exchange of DNA fragments to yield pDO531. Plasmid pDO531 contains an in-frame protein fusion between *nifL* and *lacZ*. The determination of the mRNA start of the *nifLA* operon and the ATG start codon of the *nifL* gene will be reported elsewhere⁴³.

Expression of the *nifL* and *nifH* promoters in *K. pneumoniae*

The responses of the *nifL* and the *nifH* promoters in *K. pneumoniae* to NH_4^+ , temperature and oxygen are shown in Table 2. The expression of the *nifLA* promoter was monitored using the *nifL-lacZ* protein fusion on plasmid pDO531; the parental plasmid, pDO504, was used as a control. Expression of the *nifH* promoter was monitored using a *nifH-lacZ* protein fusion^{29,42}, cloned into a phage P4 vector (ref. 29 and D.O. and F.A., in preparation) and integrated into the *K. pneumoniae* chromosome. This latter protein fusion will be described in detail elsewhere^{29,42}. Experiments 1–3 (Table 2) show that the *nifL* promoter is relatively insensitive to high temperature (37 °C) or the presence of oxygen, in contrast to the sensitivity exhibited by the *nifH* promoter. The combination of high temperature and oxygen, however, reduced expression of the *nifLA* operon considerably. Repression of the *nifLA* operon was seen in the presence of 30 mM NH_4^+ . These results corroborate previous reports on the response of the *nifLA* and *nifHDKY* operons to NH_4^+ , temperature and oxygen in *K. pneumoniae*^{4,5,8,30}.

Effect of *gln* mutations on expression of the *nifLA* operon

A model for the control of *nif* expression has been proposed in which the control elements of the general nitrogen regulatory system regulate *nif* expression through the *nifLA* operon^{1,2,5,31}.

We have tested the validity of this model by monitoring β -galactosidase synthesis from pDO531 in various *K. pneumoniae* and *E. coli* strains containing different *gln* mutations (Table 3). Experiments 9 and 10 (Table 3) show that a *glnA⁺glnG⁺* plasmid, but not a *glnA⁺glnG⁻* plasmid, complemented the *GlnA⁻GlnG⁻* *K. pneumoniae* strain KP5060 in the regulated activation of the *nifLA* promoter. Experiments 11 and 12 show further: (1) that a plasmid (pgln53Y) containing only *glnG⁺* is sufficient to activate the *nifLA* promoter in a *gln(ΔG)* *E. coli* host; and (2) that NH_4^+ does not repress the activation of *nifLA* by *glnG* product produced constitutively from pgln53Y. These results are consistent with the current model that the *glnL* product is involved in repression (refs 10, 19 and R. Bueno, G. Pahel and B. Magasanik, in preparation). This latter result is verified by the constitutive expression of *nifLA* in host YMC15, which carries the *glnL302* allele (experiments 13 and 14). The *glnL* allele has been previously shown to confer constitutive expression on other nitrogen assimilation genes (10, 22). Experiments 13 and 15 show that *glnF* is also required for *nifLA* activation, as pDO531 did not synthesize β -galactosidase in the *glnFΔ* strain TH1. Moreover, the addition of the high copy *glnA* promoter-*glnG* fusion plasmid pgln53Y did not restore activation of *nifLA* (data not shown).

Finally, a current nitrogen regulation model states that *glnD* mutant (UTase-deficient) strains do not fully activate the *glnALG* operon due to the inability to convert P_{II} (encoded by *glnB*) into its uridylylated form ($\text{P}_{\text{II}}\text{-UMP}$), and consequently, the de-uridylylated form of P_{II} remains as a co-repressor to prevent the *glnG* gene product from becoming an effective

Table 2 Expression of the *nifL* and *nifH* promoters in *K. pneumoniae*

Experiment number/ strain used	Relevant host/plasmid properties	β -Galactosidase activity							
		30 (-/-)	30 (+/+)	30 (+/-)	30 (+/+)	37 (-/-)	37 (+/+)	37 (+/-)	37 (+/+)
(1) KP5614(pVSA3)	Wild type (<i>nifH-lacZ</i> fusion)	1,639 (117)	2 (<4)	13 (<3)	2 (<3)	23 (<3)	0 (<3)	7 (<4)	3 (<4)
(2) KP5614/pDO504	Wild type/ <i>Tc^r</i> <i>nifLA</i> control	2 (100)	1 (<3)	0	1	2	1	3	1
(3) KP5614/pDO531	Wild type/ <i>nifL-lacZ</i> fusion	293 (60)	5 (<3)	77 (<3)	2 (<3)	158 (<3)	2 (<3)	14 (<3)	2 (<3)
(4) KP5614/pDO531, pWB6	Wild type/ <i>nifL-lacZ</i> fusion, <i>Ap^rTc^s</i> control	236 (100)	8	67 (3)	6	281 (<2)	1	67 (<2)	4
(5) KP5614/pDO531, pGR378	Wild type/ <i>nifL-lacZ</i> fusion, <i>nifL</i> promoter	257 (34)	25	91	22	152	1	108	9
(6) KP5614/pDO531, pACYC177	Wild type/ <i>nifL-lacZ</i> fusion, <i>Ap^r</i> control	284 (100)	18	65 (6)	15	437 (<1)	1	56 (5)	10
(7) KP5614/pDO531, pGR398	Wild type/ <i>nifL-lacZ</i> fusion, <i>nifA⁻</i>	247 (107)	22	63	17	336	1	43	9
(8) KP5614/pDO531, pGR397	Wild type/ <i>nifL-lacZ</i> fusion, <i>Km</i> promoter- <i>nifA</i> fusion	384 (162)	251	184	109	402	25	135	73

nif-derepression medium consists of NFDM medium⁴ supplemented with casamino acids (100 $\mu\text{g ml}^{-1}$), the appropriate antibiotics (tetracycline, 10 $\mu\text{g ml}^{-1}$; kanamycin, 10 $\mu\text{g ml}^{-1}$; chloramphenicol, 30 $\mu\text{g ml}^{-1}$; ampicillin, 50 $\mu\text{g ml}^{-1}$ for *E. coli*, 200 $\mu\text{g ml}^{-1}$ for *K. pneumoniae*), and further supplements for auxotrophic or vitamin-deficient strains (L-histidine, L-proline, 20 $\mu\text{g ml}^{-1}$; freshly prepared glutamine, 100 $\mu\text{g ml}^{-1}$; thiamine, 10 $\mu\text{g ml}^{-1}$). 5 ml of *nif*-derepression medium with 2 mg ml^{-1} of $(\text{NH}_4)_2\text{SO}_4$ (and 2 mg ml^{-1} of freshly prepared L-glutamine for glutamine auxotrophic strains) were inoculated with the desired strain in a plastic 12 ml conical centrifuge tube. Tubes were capped with serum stoppers and placed in a tube roller at 30 °C. At saturation, the cells were collected by centrifugation and resuspended in derepression medium with or without added $(\text{NH}_4)_2\text{SO}_4$ (2 mg ml^{-1}). For anaerobic derepression, the tubes were recapped with serum stoppers and 2 ml of the gas phase was withdrawn. For aerobic derepression, 2.5 ml of cells were transferred to 18 mm $\times$ 150 mm test tubes with loose caps. All tubes were placed in a tube roller at 30 °C or 37 °C and allowed to derepress for 10 h. In these conditions, cells begin derepressing at about 2 h ($t = 2$ h) and plateau at 8 to 10 h. Hence a $t = 10$ h measurement represents the accumulated total production of acetylene or β -galactosidase. At $t = 10$ h, 0.5 ml of cells were collected by centrifugation and resuspended in 1 ml of PS buffer (0.05 M KH_2PO_4 , 0.15 M NaCl, pH 7.2) plus 100 $\mu\text{g ml}^{-1}$ chloramphenicol (Cm) or spectinomycin (Sp), β -galactosidase assays were performed using the SDS- CHCl_3 lysis method as described in Miller⁴⁹. To verify the presence of functional nitrogenase at this stage, 2 ml of cells were diluted twofold with PS buffer with 200 $\mu\text{g ml}^{-1}$ Cm or Sp and resuspended at 30 °C anaerobically for 4 h in the presence of 2 ml of acetylene. Acetylene reduction assays were performed as described previously³³. The heading of each vertical column indicates the temperature (°C), and the presence or absence of oxygen and NH_4^+ ($\pm/\pm$). β -Galactosidase units are defined in Miller⁴⁹. Acetylene reduction values, expressed as a percentage of KP5614/pDO504 are indicated in parentheses; not all samples were determined. The values shown are from one of three independent experiments in which no significant difference was observed in the overall pattern of expression. pVSA3 is a *nifH-lacZ* protein fusion²⁹ cloned in *Km^r* P4DO105, a phage P4 plasmid vector that can integrate by P4 site-specific recombination into the *K. pneumoniae* chromosome (ref. 28 and our manuscript in preparation). KP5614 (pVSA3) was constructed by transformation selecting for *Km^r* transformants. Its integrated form has been verified by Southern blot hybridization and genomic DNA with P4DO105 as DNA probe (manuscript in preparation).

activator (refs 23, 32 and R. Bueno, G. Pahel and B. Magasanik, in preparation). Thus, according to this model, *glnD* mutant strains are unable to activate nitrogen assimilation genes such as *hut* and *aut* due to insufficient levels of the *glnG* product. Consistent with this model, the *nifL-lacZ* fusion is unable to synthesize β -galactosidase in the *glnD* Δ strain, DO1413 (experiments 13 and 16, Table 3). In contrast to the *glnF* Δ strain, however, the addition of the high copy *glnG* plasmid pgn53Y to DO1413 fully restored the constitutive activation of *nifLA* (experiments 17 and 18). Presumably, high levels of the *glnG* gene product can overcome the repressing effects of de-uridylylated P_{II}. It is also possible that the de-uridylylated form of P_{II} could be antagonizing the *glnL* gene product, thereby allowing DO1413/pDO531, pgn53Y to

escape NH₄⁺ repression (R. Bueno, G. Pahel and B. Magasanik, in preparation). In all respects examined thus far, the transcriptional regulators of nitrogen assimilation control the *nifL* promoter in exactly the same fashion as described for other nitrogen assimilation pathways.

It has been shown that the activator of the *nifHDKY* operon (*nifA*) is present in limiting quantity and can be titrated away from a chromosomal *nifH* promoter by high copies of the *nifH* promoter on a multicopy plasmid^{6,33}. We therefore investigated whether an analogous case exists between *gln*-activating elements and the *nifL* promoter. Table 2, experiments 4 and 5, show that multiple copies of pGR378, a pBR322 derivative that carries the *nifL* promoter and which is compatible with pDO531, did not significantly affect the activation of the *nifL*

Table 3 The effect of *gln* mutations on the activities of the *nifL* and *glnA* promoters

Experiment no./strain used	Relevant host/plasmid properties	Units of β -galactosidase	
		-NH ₄ ⁺	+NH ₄ ⁺
(9) KP5060/pDO531, pFB54	<i>glnA</i> ⁻ <i>G</i> ⁻ / <i>nifL-lacZ</i> fusion, <i>glnA</i> ⁺	2 [†]	3
(10) KP5060/pDO531, pFB514	<i>glnA</i> ⁻ <i>G</i> ⁻ / <i>nifL-lacZ</i> fusion, <i>glnA</i> ⁺ <i>G</i> ⁺	126	4
(11)* FDB213/pDO531, pWB6 [‡]	<i>gln</i> (ALG) Δ / <i>nifL-lacZ</i> fusion, Ap ^r Tc ^r control	6	6
(12)* FDB213/pDO531, pgn53Y	<i>gln</i> (ALG) Δ / <i>nifL-lacZ</i> fusion, <i>glnA</i> promoter- <i>glnG</i> ⁺ fusion	228	354
(13)* YMC10/pDO531	<i>lac</i> Δ / <i>nifL-lacZ</i> fusion	140	1
(14)* YMC15/pDO531	<i>lac</i> Δ <i>glnL</i> 302/ <i>nifL-lacZ</i> fusion	310	126
(15)* TH1/pDO531	<i>lac</i> Δ <i>glnF</i> Δ / <i>nifL-lacZ</i> fusion	1	<1
(16)* DO1413/pDO531	<i>lac</i> Δ / <i>nifL-lacZ</i> fusion	1	2
(17)* DO1413/pDO531, pWB6	<i>lac</i> Δ <i>glnD</i> Δ / <i>nifL-lacZ</i> fusion, Ap ^r Tc ^r control	1	1
(18)* DO1413/pDO531, pgn53Y	<i>lac</i> Δ <i>glnD</i> Δ / <i>nifL-lacZ</i> fusion, <i>glnA</i> promoter- <i>glnG</i> ⁺ fusion	843	718
(19) NK6986/ <i>F'lacI</i> ^Q , pDO516, pDO531 (-IPTG) [§]	<i>lac</i> Δ / <i>lacI</i> ^Q , <i>lacPO-nifA</i> fusion, <i>nifL-lacZ</i> fusion	70	1
(20) NK6986/ <i>F'lacI</i> ^Q , pDO516, pDO531 (+IPTG)	<i>lac</i> Δ / <i>lacI</i> ^Q , <i>lacPO-nifA</i> fusion, <i>nifL-lacZ</i> fusion	89	174
(21)* YMC11/ <i>F'lacI</i> ^Q , pDO516, pDO531 (-IPTG)	<i>lac</i> Δ <i>gln</i> (ALG) Δ / <i>lacI</i> ^Q , <i>lacPO-nifA</i> fusion, <i>nifL-lacZ</i> fusion	19	25
(22)* YMC11/ <i>F'lacI</i> ^Q , pDO516, pDO531 (+IPTG)	<i>lac</i> Δ <i>gln</i> (ALG) Δ / <i>lacI</i> ^Q , <i>lacPO-nifA</i> fusion, <i>nifL-lacZ</i> fusion	104	106
(23)* DO1413/ <i>F'lacI</i> ^Q , pDO516, pDO531 (-IPTG)	<i>lac</i> Δ <i>glnD</i> Δ / <i>lacI</i> ^Q , <i>lacPO-nifA</i> fusion, <i>nifL-lacZ</i> fusion	16	16
(24)* DO1413/ <i>F'lacI</i> ^Q , pDO516, pDO531 (+IPTG)	<i>lac</i> Δ <i>glnD</i> Δ / <i>lacI</i> ^Q , <i>lacPO-nifA</i> fusion, <i>nifL-lacZ</i> fusion	42	59
(25)* TH1/ <i>F'lacI</i> ^Q , pDO516, pDO531 (-IPTG)	<i>lac</i> Δ <i>glnF</i> Δ / <i>lacI</i> ^Q , <i>lacPO-nifA</i> fusion, <i>nifL-lacZ</i> fusion	14	14
(26)* TH1/ <i>F'lacI</i> ^Q , pDO516, pDO531 (+IPTG)	<i>lac</i> Δ <i>glnF</i> Δ / <i>lacI</i> ^Q , <i>lacPO-nifA</i> fusion, <i>nifL-lacZ</i> fusion	44	58
(27)* YMC9(λ gln101) /pGR398	<i>lac</i> Δ (<i>glnA</i> promoter- <i>lacZ</i> fusion)/ <i>nifA</i> ⁻	1,658 (69) [¶]	264 (14)
(28)* YMC9(λ gln101)/pGR397	<i>lac</i> Δ (<i>glnA</i> promoter- <i>lacZ</i> fusion)/Km promoter- <i>nifA</i> fusion	1,586 (71)	513 (28)
(29)* YMC17(λ gln101)/pGR398	<i>lac</i> Δ <i>glnG</i> :: Tn5 (<i>glnA</i> promoter- <i>lacZ</i> fusion)/ <i>nifA</i> ⁻	83 (5.4)	91 (4.2)
(30)* YMC17(λ gln101)/pGR397	<i>lac</i> Δ <i>glnG</i> :: Tn5 (<i>glnA</i> promoter- <i>lacZ</i> fusion)/Km promoter- <i>nifA</i> fusion	1,315 (68)	585 (37)
(31) DO1413(λ gln101)/pGR398	<i>lac</i> Δ <i>glnD</i> Δ (<i>glnA</i> promoter- <i>lacZ</i> fusion)/ <i>nifA</i> ⁻	215 (7.3)	97 (2.4)
(32)* DO1413(λ gln101)/pGR397	<i>lac</i> Δ <i>glnD</i> Δ (<i>glnA</i> promoter- <i>lacZ</i> fusion)/Km promoter- <i>nifA</i> fusion	1,856 (76)	802 (40)
(33)* TH1(λ gln101)/pGR398	<i>lac</i> Δ <i>glnF</i> Δ (<i>glnA</i> promoter- <i>lacZ</i> fusion)/ <i>nifA</i> ⁻	48	57
(34)* TH1(λ gln101)/pGR397	<i>lac</i> Δ <i>glnF</i> Δ (<i>glnA</i> promoter- <i>lacZ</i> fusion)/Km promoter- <i>nifA</i> fusion	49	59
(35)* ET6362(λ gln101)/pGR398	<i>lac</i> Δ <i>glnF</i> :: Tn10 <i>glnG</i> :: Tn5 (<i>glnA</i> promoter- <i>lacZ</i> fusion)/ <i>nifA</i> ⁻	137	156
(36)* ET6362(λ gln101)/pGR397	<i>lac</i> Δ <i>glnF</i> :: Tn10 <i>glnG</i> :: Tn5 (<i>glnA</i> promoter- <i>lacZ</i> fusion)/Km promoter- <i>nifA</i> fusion	119	124

* Derepression conditions at 30 °C as described in Table 2 legend except that cells were grown to saturation with the additional supplement of 2 mg ml⁻¹ L-glutamine and resuspended with an additional 100 μ l ml⁻¹ L-glutamine. This is because glutamine-auxotrophic strains require high levels of glutamine for growth but can be derepressed after saturated growth in low levels of glutamine (100 μ g ml⁻¹). To show that the low level of glutamine does not prevent the synthesis of β -galactosidase because of lack of protein synthesizing substrates, a *glnA*⁻*lacZ*⁺ *E. coli* strain, FDB213, was tested for its ability to synthesize β -galactosidase. After saturated growth with 0.2% glutamine, the cells were resuspended in fresh media with 100 μ g ml⁻¹ of glutamine and 2% glycerol instead of the usual 2% glucose in order to avoid catabolite repression of the *lac* operon. When the *lac* inducer isopropyl- β -D-thiogalactoside (IPTG) was added to the derepressed culture (2.5 mM), over 1,000 units of β -galactosidase were synthesized (data not shown).

[†] β -galactosidase units as defined in Miller⁴⁹. Derepression conditions performed as described in Table 2 legend. The values shown are from one of two independent experiments in which no significant difference was observed in the overall pattern of expression.

[‡] pWB6 is used as a Tc^rAp^r pBR322 control plasmid.

[§] *F'lacI*^Q indicates the *F'lacI*^Q*lacZ* :: Tn5 *pro*⁺ episome from a standard mating with donor strain NK7047. + or - IPTG indicates that cells were resuspended in derepression media supplemented with or without 2.5 mM isopropyl- β -D-thiogalactoside, respectively.

^{||} λ gln101 lysogens of YMC9, YMC17, TH1, DO1413, and ET6362 were made as described by Backman *et al.*²¹.

[¶] Glutamine synthetase activity in nmol of glutamyl hydroxamate per min per ml of cells. The γ -GT assay described by Bender *et al.*⁵⁰ was used to measure the total amount of glutamine synthetase present.

promoter on pDO531. We conclude that the *gln*-activating elements (*glnG* and *glnF*) are not present in limiting quantities. Interestingly, however, the *nifL* promoter on pDO531 appeared less sensitive to repression in the presence of pGR378. Perhaps elements that mediate repression of the *nifL* promoter may be limiting.

The *nifA* gene product activates its own promoter

Although the *nifA* gene product may not be required for *nifLA* transcription, it is conceivable that activation of this operon may be enhanced by the *nifA* gene product and thereby alleviate the requirement for high levels of *glnG* and possibly *glnF* gene products. We investigated this possibility using both constitutive and inducible *nifA*-overproducing plasmids. The constitutive *nifA*-overproducing plasmid (pGR397) consisted of a transcriptional fusion between the kanamycin promoter of pACYC177 and *nifA*. The same *nifA* fragment cloned in the opposite orientation (pGR398), which does not produce high levels of the *nifA* product, served as a control. An inducible *nifA*-overproducing plasmid (pDO516) was also constructed in which *nifA* was transcriptionally fused to the *E. coli* *lacPO*_{UV5} promoter (the UV5 mutation renders the *lac* promoter insensitive to glucose repression)³⁴. Repression of the *lacPO*_{UV5} promoter on the multicopy plasmid pDO516 was accomplished by the use of a repressor-overproducing gene *lacI*^Q on the episome *F'**lacI*^Q*lacZ* :: Tn5*pro*⁺. In this latter strain, *nifA* transcription is only switched-on in the presence of a *lac* inducer, such as isopropyl-β-D-thiogalactoside (IPTG). Experiments 6–8 (Table 2) show that, whereas the control plasmids pACYC177 and pGR398 did not affect the expression of the *nifL* promoter in the *K. pneumoniae* host, pGR397 enhanced *nifL*-promoter activity in all physiological conditions examined. Most notable is the circumvention of normal repression by NH₄⁺ and, to a lesser extent, repression by oxygen and high temperature. When an analogous experiment was performed in an *E. coli* host (Table 3, experiments 19, 20), NH₄⁺ repression was also overridden when *nifA* was derepressed by the addition of IPTG.

We next attempted to distinguish between the possibilities that activation of the *nifLA* promoter is mediated directly by the *nifA* gene product or indirectly through the activation of *gln* regulatory elements (for example by activation of the *glnALG* operon). Experiments 21 and 22 show that overproduction of the *nifA* gene product activated the *nifL* promoter in the absence of the *glnALG* operon. Experiments 23 and 24 show a similar effect in the absence of a functional *glnD* gene, where low levels of *glnG* gene product are presumably present¹⁰. Moreover, the absence of the *glnF* gene (experiments 25, 26) did not prevent *nifA* activation of its own promoter. In the cases of a *glnFΔ* or a *glnDΔ* strain, however, there appeared to be an effect on the levels of expression. In the *glnFΔ* strain, one possibility is that *glnF* may be a contributor to *nifA* activation as seen in the case of *nifA* activation of the *glnA* promoter (see below). In the *glnDΔ* host, it is possible that P_{II} may interact directly or indirectly with the *nifA* protein because of its similarity to the *glnG* gene product (see below). We conclude from these experiments that high levels of the *nifA* gene product can activate its own transcription unit in the absence of the *glnG* product or the *glnF* product. Whether the *glnF* product a role in enhancing transcriptional activation (in conjunction with the *nifA* product) is less certain.

The *nifA* gene product activates the *glnALG* promoter

We examined the possibility that the *nifA* gene product activates the *glnALG* promoter (and thereby indirectly activates its own *nifLA* promoter by mediating higher levels of *glnG* proteins) with the use of λgln101, which contains the *glnA* promoter transcriptionally fused to *lacZ* and cloned in phage λ as described by Backman *et al.*²¹. Experiments 27 and 28 show only slight enhancement of the *glnALG* promoter in the

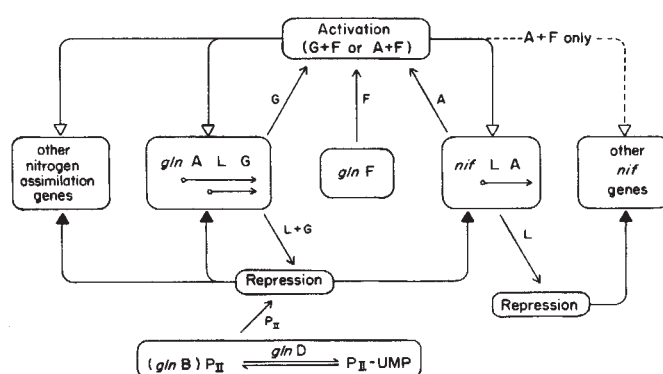


Fig. 3 A revised model of *nif* regulation. (⇓) and (⇑) signify activation and repression pathways, respectively. On nitrogen starvation, *glnG* and *glnF* activate the *glnALG* transcription unit. This results in higher levels of glutamine synthetase (which has a major role in NH₄⁺ assimilation) as well as higher levels of the *glnG* product itself. *glnG* and *glnF* also activate other nitrogen assimilatory genes such as those involved in the degradation and transport of amino acids, depending on the availability of these nitrogen sources. In addition, the *nifLA* operon is activated. Under conditions in which nitrogen remains limiting and in which the oxygen tension is low, the *nifA* product autoactivates its own transcription unit and other *nif* transcription units. To facilitate efficient NH₄⁺ assimilation, the *nifA* product also activates the *glnA* promoter.

wild type strain by the overproduction of *nifA* under NH₄⁺ repression. However, in a *glnG* :: Tn5 or a *glnDΔ* background, we observed a wild type level of activation from the *glnA* promoter in the *nifA* constitutive strain, indicating that the *nifA* gene product can substitute for the *glnG* product *in vivo* (experiments 29–32). Corresponding glutamine synthetase levels confirm this observation (experiments 27–32). Furthermore, as was observed in the activation of the *glnA* promoter by the *glnG* product^{13,17,18}, a requirement for the *glnF* gene product is necessary for the activation of the *glnA* promoter by *nifA* (experiments 33, 34). From the pattern of *glnALG* activation described above, it appears that *nifA* can substitute for *glnG* with apparently the same requirement for *glnF* as previously reported for *glnG*.

An alternative possibility can explain the involvement of *glnF* in *nifA*-mediated gene activation. The *glnG* product has been suggested to be a negative regulator for the *glnALG* operon^{19,20}. It has also been implicated in the negative regulation of the ammonia-dependent asparagine synthetase gene *asnA*³⁵. Consequently, it is possible that in a *glnF* defective background, *glnG* product may be 'locked' into a repressor mode, and the *nifA* product may be unable to derepress a *glnG* repressed promoter. This seems unlikely because experiments 35 and 36 show that pGR397 also cannot activate the *glnALG* promoter in the *glnG* :: Tn5 *glnF* :: Tn10 strain, ET6362. Consistent with this result, ET6362 harbouring pGR397 or pDO516 cannot grow with arginine or proline as sole nitrogen source (see below). Thus, the *glnF* gene appears to be involved in *nifA*-mediated activation in a more direct fashion. In addition to the data reported here, we recently found (in collaboration with V. Sundaresan) that activation of the *nifH* promoter requires both the products of *nifA* and *glnF*. The data to support this latter result will be published separately. (In experiments 27–36, work was also conducted with pACYC177 as a control plasmid. As results obtained with either pACYC177 or pGR398 did not differ, the data for pACYC177 are not shown.)

Finally, experiments 30 and 32 show that *nifA*-mediated expression from the *glnA* promoter is still partially regulated by NH₄⁺. As *glnG* appears to be involved in NH₄⁺ repression of the *glnA* promoter^{19,20}, it is possible that *nifA* not only substitutes for *glnG* in its role as an activator, but may also be involved in NH₄⁺ repression of the *glnA* promoter (along with the other *gln* co-repressors). In contrast, NH₄⁺ repression of the *nifL* promoter was not observed (experiments 19–24).

Perhaps *nifL*, known also to mediate NH_4^+ repression, is an additional factor required for the repression of its own operon. Alternatively, this effect may be due to either higher levels of *nifA* produced by pDO516 as opposed to pGR397 or to a lower affinity of the *nifA* product for the *glnA* promoter in the presence of NH_4^+ .

The *nifA* gene product activates other nitrogen utilization genes

The finding that the *nifA* product can replace the *glnG* product in activating both its own promoter and that of the *glnALG* operon prompted us to investigate whether the *nifA* product can activate other genes positively controlled by *glnG* such as those governing the catabolism of certain amino acids. We found that the *E. coli glnG::Tn5* and *glnDΔ* strains YMC17 and DO1413 respectively, recovered the ability to use arginine or proline as sole nitrogen source when harbouring the *nifA* overproducing plasmid pGR397, but not the control plasmids pGR398 or pACYC177. Similarly, the *K. aerogenes glnG::Tn5* strain DO1490 grows like the wild-type parent CG566 with histidine or asparagine as sole source of nitrogen (NFD medium with 0.4% glucose, 0.2% nitrogen source) when harbouring pGR397, but not pGR398 nor pACYC177. We conclude that the *nifA* product can replace the *glnG* product in the activation of these *glnG* positively controlled genes.

Conclusions

The evidence we have presented demonstrates that the *nifA* product produced from a multicopy plasmid can substitute for the *glnG* product in its role as a general nitrogen assimilation pathway regulator, and leads to the suggestion that *nifA* and *glnG* may be evolutionarily related. In support of this hypothesis, we point to the following facts: (1) Both the *glnG*^{19,21} and *nifA*^{36,37} products have a molecular weight of approximately 55,000. (2) Both the *nifLA* and *glnLG* operons (*glnLG* is a sub-operon of the larger *glnALG* transcription unit^{20,38} code for products involved in repression and activation in the same physical arrangement. (3) Both *nifL* and *glnL* mediate NH_4^+ repression. (4) In enterobacteria, statistical analyses have shown that metabolically related genes are likely to be located 90° or 180° apart on the circular chromosome, and have led to the proposal that the chromosomes of present day enterobacteria have undergone two duplications (for review, see ref. 39). In this context, it is interesting that the *glnALG* operon and the *his* operon map at 86 min and 44 min respectively, on the *E. coli* genetic map⁴⁰. Since the genetic maps of *E. coli* and *K. pneumoniae* are similar⁴¹, this would imply that the *his*-linked *nif* cluster is situated approximately 180° apart from the *glnALG* operon. All these facts suggest that the *nifLA* operon may have descended from an ancestral *glnLG* unit.

Although the *nifA* product can substitute for the *glnG* product in activating several different nitrogen assimilation operons, the reverse is not true with respect to the *nif* operons. That is, in *K. pneumoniae*, all *nif* promoters, except the *nifLA* promoter, appear to respond only to functional *nifA* activator (and perhaps only to *nifL* repressor), and not to *glnG* activator. The explanation for this feature of the *nif* control system may be related to the fact that *K. pneumoniae* is a free-living organism, which must be able to respond quickly to a constantly changing environment. It is possible, therefore, that the evolution of the *nifLA* operon in *K. pneumoniae* may have served to mediate more stringent control on the expression of an extremely oxygen-sensitive, energy-intensive, biochemical process. If this hypothesis is correct, one might expect that in species that fix nitrogen under more controlled environmental conditions than *K. pneumoniae*, that *glnF* and *glnG* would act directly at the *nif* promoters. In fact, in the case of the symbiotic nitrogen-fixing species *Rhizobium meliloti*, in which nitrogenase genes are normally only expressed in the protected environment of the root nodule, we have shown that the *R. meliloti nifH* promoter responds to activation by *E. coli glnG* in conjunction with *E. coli glnF*⁴². The *R. meliloti nifH* promoter also responds

to activation by *K. pneumoniae nifA*, providing further evidence for the close evolutionary relationship of *nifA* and *glnG*²⁹.

Additional observations and arguments are consistent with the hypothesis that the *nifLA* operon evolved from an ancestral *glnLG* operon: (1) The *nifL* protein (molecular weight 45,000, 45K), which has both NH_4^+ and oxygen monitoring functions, is larger than the *glnL* protein (34K), which appears to respond only to NH_4^+ . (2) A physiological advantage of duplicating the functions of *glnG* may be to alleviate the high level demand for *glnG*. By autoactivating its own operon (as well as the *glnALG* operon), *nifA* may be amplifying the activation of *nif* expression initiated by *glnG*. (3) It is possible that *nifA* may act on the *glnALG* operon not only to promote high levels of *glnG* product but also of *glnA* product. The *glnA* gene encodes the enzyme glutamine synthetase that catalyses the major pathway of NH_4^+ assimilation into glutamine. (It is virtually the sole operative pathway under low NH_4^+ concentrations⁹.) *nifA*-mediated activation of the *glnALG* operon during nitrogen fixation may thereby prevent repression of the *glnALG* operon by NH_4^+ , and may facilitate maximum efficiency in assimilating NH_4^+ and consequently in fixing nitrogen. In contrast, this role of *nifA* may not be required in nitrogen-fixing cells that excrete free NH_4^+ ions, such as in the case of the *Rhizobium*-plant symbiosis⁴⁴.

As noted earlier, *nifA*-mediated activation of the *glnALG* promoter is still subjected to NH_4^+ repression. We have suggested that *nifA* may also be substituting for *glnG* in its involvement in negative regulation of the *glnALG* promoter. It is interesting to speculate that *nifA* may perform a similar role for the *nif* operons. Concerning the role of *glnF*, it is not known whether it has a direct role as a co-activator in concert with the *glnG* product, or an indirect role by activating *glnG* protein synthesis or by catalytically converting the *glnG* product into an activator form. Its presence appears to be required for the *nifA* product to mediate full activation of the *glnALG* operon and genes governing the utilization of arginine, proline, histidine and asparagine. It may also be required to mediate higher levels of expression of the *nifLA* operon. C. Elmerich and L. Sibold (personal communication) have also observed that *glnF* product is involved in *nifA*-mediated activation.

In addition to the data presented in this manuscript, we have determined the DNA sequences and transcription start sites for three promoters regulated by *nifA* gene product (see accompanying article⁴⁵). They are the *K. pneumoniae nifL* and *nifH* promoters and the *R. meliloti nifH* promoter^{42,43}. The *K. pneumoniae nifL* and *R. meliloti nifH* promoters are also regulated by *glnG*. Comparison of the DNA sequences of these two *glnG* regulated promoters, and of three other promoters (*E. coli glnA* promoter and the *Salmonella typhimurium dhuA* and *argTr* promoters) also regulated by *glnG* reveals a consensus sequence of TTTTGCA. For the *nif* promoters, where the transcriptional start sites have been mapped, this sequence was found at the -15 region. In contrast, the *K. pneumoniae nifH* promoter, which is not activated by *glnG* but by *nifA*, does not have this consensus sequence.

Based on our data, we propose the *nif* regulation model summarized in Fig. 3, which includes regulatory pathways found in this study, although direct evidence for their physiological significance has yet to be demonstrated. However, even a minute physiological difference (perhaps not even detectable within 30 generations of laboratory growth) may be significant evolutionarily when millions of generations are considered. Results similar to ours concerning the similarity of *glnG* and *nifA*, have recently been attained by Merrick⁴⁶.

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LETTERS TO NATURE

The 1.5 ms pulsar PSR1937+21

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At 1.55 ms the period (P) of the newly discovered pulsar PSR 1937+21 in 4C21.53 (ref. 1) is two or three orders of magnitude smaller than most other pulsars. We have measured the rate of change of period and found it to be also remarkably small, so that the pulsar cannot be on the same evolutionary track as any other previously known. Our measured value of $\dot{P}$ is $(1.7 \pm 0.7) \times 10^{-19}$, which is four orders of magnitude below the previously quoted upper limit². The formal error depends heavily on the accuracy of the assumed pulsar position: moreover, recent work suggests that there may be a systematic error which affects this position, and the correct value of $\dot{P}$ is probably as low as $(0.4 \pm 0.7) \times 10^{-19}$. Note therefore that even our present measurements may ultimately be providing an upper limit for $\dot{P}$. We present here measured characteristics of this pulsar which suggests that it is nevertheless behaving like many others. The lifetime is at least 10^8 yr, and the magnetic dipole moment must be a factor of about 10^4 lower than in the Crab Pulsar. The pulsar is not a member of a binary system.

We used the 75-m Mk 1A telescope at 1,667 MHz for a total of 60 h between 23 November and 12 December 1982. The receiver bandwidth of 4 MHz was divided into 32 channels each 125 kHz wide which were separately delayed to compensate for interstellar dispersion. Coherent integrations lasting 1 or 2 s were recorded on magnetic tape and used to determine an accurate value of period which was then used in co-adding 300 s of recording. Standard techniques for reducing the arrival times to the barycentre, and frequent references to the Loran time service, allowed us to work to an accuracy of ~ 1 μ s.

Over the entire period of 19 days which included over 10^9 pulses we were able to follow the phase of the pulses to within $\sim 3\%$ of P . Furthermore, this fit was achieved by setting $\dot{P}$, the rate of change of period, to zero. The fit is shown in Fig. 1. The small curvature indicated by the solid line represents the best fit value of $\ddot{P}$. The exact values of P and $\dot{P}$ depend on the assumed position of the pulsar. The timing results are presented in Table 1, which gives the results of the fit obtained using the VLA position given by Backer *et al.*¹ together with the partial derivatives of P and $\dot{P}$ with respect to small changes in right

ascension and declination. The formal fitting errors are given for P and $\dot{P}$ as well as the total errors computed assuming uncertainties of 0.5 arc s in both right ascension and declination.

Note that the total error in our value of P is dominated by the positional uncertainty. There is some evidence (E. Fomalont, M. Goss, A.G.L. and R. N. Manchester, in preparation) of a systematic difference between the positions of pulsars determined using the VLA and from arrival time observations, in which the positions are those required to remove any annual sinusoidal variation in the residual arrival times at the Solar System barycentre. In the vicinity of PSR1937+21, the timing positions appear to be systematically offset from the VLA positions by about +0.75 arc s and +0.6 arc s in right ascension and declination. If offsets of these amounts are applicable to PSR1937+21 the value of $\dot{P}$ becomes $(0.4 \pm 0.7) \times 10^{-19}$ indicating that the measured value is probably an upper limit. The curvature in Fig. 1 may then be part of the residual annual sinusoid which will arise because of the incorrect reduction of the arrival times to the barycentre. To obtain a substantially improved estimate of $\dot{P}$, observations over a period of about 1 yr are required.

This value of $\dot{P}$ is a factor of 10^4 lower than the upper limit provided by Backer *et al.*² and indicates a characteristic age ($P/2\dot{P}$) of at least 1.5×10^8 yr. Assuming a moment of inertia of 10^{45} g cm², the surface magnetic field $(3.2 \times 10^{19} (P\dot{P})^{1/2})$ (ref. 3) is less than 5×10^8 G. The r.m.s. residual after fitting for P and $\dot{P}$ is 6 μ s and the lack of any periodic modulation rules out any possibility that this pulsar is a member of a close binary system.

Table 1 Timing results and rate of change of P

Assumed RA (1950.0)	19 h 37 min 28.72 $\pm$ 0.03 s
Assumed Dec (1950.0)	21°28'1.3" $\pm$ 0.5
Epoch JD 244 5306.5	
Barycentric period P	1 557 806 449 059 $\times 10^{-9}$ μ s
Fitting error	4 $\times 10^{-9}$ μ s
Total error	339 $\times 10^{-9}$ μ s
$\partial P / \partial (\text{RA cos Dec})$	-567 $\times 10^{-9}$ μ s arc s ⁻¹
$\partial P / \partial \text{Dec}$	+290 $\times 10^{-9}$ μ s arc s ⁻¹
Period derivative $\dot{P}$	1.67 $\times 10^{-19}$
Fitting error	0.13 $\times 10^{-19}$
Total error	0.71 $\times 10^{-19}$
$\partial \dot{P} / \partial (\text{RA cos Dec})$	-1.08 $\times 10^{-19}$ arc s ⁻¹
$\partial \dot{P} / \partial \text{Dec}$	-0.89 $\times 10^{-19}$ arc s ⁻¹

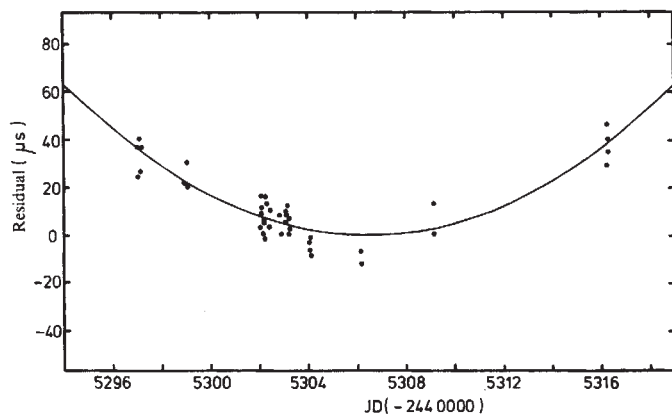


Fig. 1 The residuals in arrival time at the barycentre of the Solar System over a period of 19 days obtained by assuming a constant barycentric period. The curved line is the expected variation for a period derivative of $1.67 \times 10^{-19} \text{ s s}^{-1}$.

Other characteristics of this pulsar are closer to normal. Figure 2 shows the pulse profile which is remarkably similar to that of the Crab pulsar. The interpulse (the weaker of the two pulses) occurs 173° of longitude before the main pulse, as compared with 215° for the Crab pulsar. The width of the main pulse, after making allowance for the effects of dispersion in the receiver, is $40 \pm 10 \mu\text{s}$ between half intensity points, equivalent to 9° of longitude, and slightly less for the interpulse. The simplest interpretation of this is that the pulsar is very nearly an orthogonal rotator, that is the magnetic axis is perpendicular to the rotation axis, and the Earth lies in the plane of the rotational equator. We note, however, that it is difficult to reconcile this model with the polarization data, also shown in Fig. 2, as this shows that the position angle of the linear polarization differs by $\sim 90^\circ$ between the main pulse and the interpulse. Nevertheless, the general polarization characteristics are very similar to those of many other pulsars whose periods are two or three orders of magnitude greater.

We find a dispersion measure of $70.5 \pm 0.3 \text{ pc cm}^{-3}$, indicating a distance of $\sim 2 \text{ kpc}$. The low galactic latitude ($b = 0.3^\circ$) places the pulsar within 20 pc of the galactic plane. We note, however, that, using the relationship established by Lyne and Smith⁴, the scintillation characteristics quoted by Backer *et al.*¹ give a strong indication that the transverse velocity is about 70 km s^{-1} which is typical of many pulsars. At such a speed the pulsar is likely to have left the vicinity of the plane well within its present characteristic lifetime: we can safely assume that its true age is very much smaller.

Despite the very low values of P and $\dot{P}$, we see no reason to assign unusual physical characteristics to this pulsar, apart from a low value of dipole magnetic moment which must be a factor of 10^4 lower than that of young pulsars such as the Crab pulsar. The rotational energy is nevertheless 400 times greater than that of the Crab pulsar, and even the very small observed slowdown rate corresponds to a total energy loss of $2 \times 10^{36} \text{ erg s}^{-1}$. This may be compared with $5 \times 10^{38} \text{ erg s}^{-1}$ for the Crab pulsar and $7 \times 10^{36} \text{ erg s}^{-1}$ for the Vela pulsar. At a distance of 2 kpc , luminosities of pulsed emission substantially less than this should be detectable up to γ -ray frequencies so that despite the small slowdown rate there is evidently a good possibility of observing pulsed optical, X-ray and γ -ray radiation from this pulsar.

The origin and history of the pulsar must be substantially different from all others except possibly the binary pulsar PSR1913+16, which also has a low value of P and $\dot{P}$. It has been suggested that PSR1913+16 is a comparatively old pulsar which has been spun up during a period of mass transfer in a binary system⁵. If the same suggestion applies to the 1.5 ms pulsar we must assume that in this case the binary system was subsequently disrupted by the supernova explosion of the companion star. The pulsar is now in the remarkable condition of

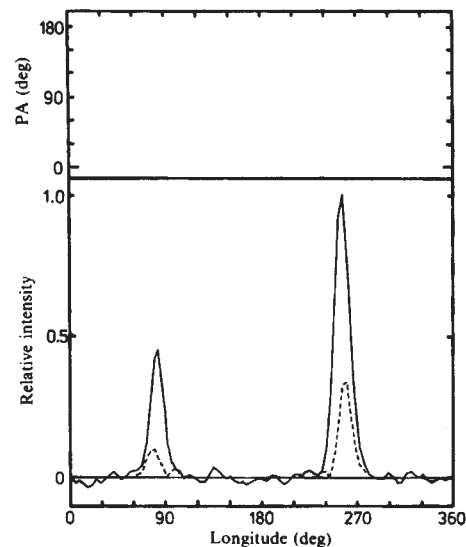


Fig. 2 The pulse shape and linear polarization of PSR1937+21 measured at $1,667 \text{ MHz}$. The total intensity and linearly polarized power are represented by the full and dashed lines respectively. The circular polarization amounted to less than 4% of the peak total intensity.

containing a phenomenal amount of rotational energy which it has no effective means of losing.

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Timing observations of the millisecond pulsar

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Following the discovery of the rapidly spinning pulsar 1937+214 on 7 November 1982¹ a program of accurate pulse arrival time measurements was initiated at the Arecibo Observatory. These observations, which were conducted at $1,408 \text{ MHz}$ centre frequency, indicate that pulse arrival times follow a simple spindown equation with an accuracy of $1.5 \mu\text{s}$ for intervals between 2 min and 14 days. The apparent period derivative of $1.2 \times 10^{-19} \text{ s s}^{-1}$ leads to a characteristic time scale $P/\dot{P}$ for period evolution of $4 \times 10^8 \text{ yr}$. If the observed stability of this pulsar persists for decades, we will be able to reduce present uncertainties on the masses of the distant planets.

After the discovery of 1937+214 we began a series of observations to determine the stability of barycentric pulse periods. We thought that large variations in the period might be observable since this star is rotating near the breakup rate for neutron stars. The radio intensity in 250 kHz bands for both circular polarizations at $1,407$ and $1,417 \text{ MHz}$ were summed and sampled continuously at $50 \mu\text{s}$ intervals. These samples were recorded on magnetic tape and then folded modulo the apparent pulse period in a computer. Apparent periods from 15-min integrations were corrected to barycentric periods by removing the Doppler factor from the Earth's rotation about the Sun. Observations from 8 to 26 November indicated that

there were no variations in the period greater than 3×10^{-7} s. The mean period was 1.557 806 5 ms and an upper limit to the period derivative was 3×10^{-16} s s⁻¹.

A second phase of pulse timing began at Arecibo Observatory on 27 November using hardware and software developed for timing observations of the binary pulsar 1913+16 (ref. 2). This system will be described only briefly here. The signals from both polarization channels are summed and passed through a 32-channel digital dedisperser³ to remove the effects of interstellar propagation. The total bandwidth of the system is 8 MHz. The dedispersed signal is passed to a signal averager which samples the data continuously with precisely 64 samples per apparent period. The time of the first sample of a period near the midpoint of each 2-min integration is recorded with an uncertainty of 0.3 μ s with respect to the observatory rubidium clock. The topocentric arrival times for each 2-min average are computed using the standard technique of template matching². Topocentric arrival times are converted to barycentric arrival times using interpolations of the Lincoln Lab ephemeris for the Earth position⁴, and our VLA position for the pulsar. The barycentric arrival times are fit by the method of least squares to a simple equation of condition for pulsar phase including a constant spin rate and its first derivative. Results from this fit are given in Table 1 along with a recent determination of the dispersion measure and the VLA position from ref. 1.

The position quoted in Table 1 comes from three 20-cm synthesis maps of 1937+214 at the VLA in its highest resolution A configuration. The duration of each observation was 8 min. The beam formed in all cases is sufficient to resolve nearly all the flux from the extended H II region north of the pulsar¹. The data were calibrated by observations of 1923+210 whose position has been determined by Perley with an accuracy of 0.05 arc s (ref. 5). The agreement of our independent measurements and the general phase stability of the VLA lead us to quote the 0.2 arc s position error in Table 1.

The errors in period and period derivative solely reflect the position uncertainties presented above. The internal errors from the fit are 1×10^{-15} s and 2×10^{-21} s s⁻¹ for the period and period derivative, respectively. The accuracy of the period derivative cannot be improved without an improved position measurement. In fact the error in our spindown determination may be larger if there are discrepancies between positions determined from pulse timing and those determined by radio interferometry. A recent comparison between timing and radio interferometric positions indicates that the two measurements agree at the 0.1 arc s level for pulsars near 1937+214 (Fomalont, personal communication). The measured period derivative could be dominated by spin 'noise' with a time scale exceeding two weeks. Observations over the coming year will allow a clear separation of effects.

The residuals from our fit of 2-min averages recorded over 2 weeks from 28 November to 12 December have an r.m.s. residual of 1.5 μ s. There is no indication of intrinsic noise in the pulse arrival times down to this level. We are in the process of discovering and removing small imperfections in the recording and analysing system to reach the estimated signal-to-noise limit of 1 μ s.

The apparent period derivative, 1.2×10^{-19} s s⁻¹, indicates a characteristic age for spindown by electromagnetic torque (EMT) of 2×10^8 yr. This value is close to predictions based on the absence of an X-ray nebula⁶⁻⁸. If the present period is close to the original period, then the EMT age is $4 \times 10^8 \Delta P/P$ yr, where $\Delta P/P$ is the fractional change in period during the

pulsar lifetime. An independent age estimate for 1937+214 comes from its proximity to the galactic plane, 0.3°. This suggests that the progenitor of the pulsar was a member of an extreme Population I class of stars such as the massive OB stars. We would then assume a scale height for the pulsar progenitor of 100 pc. If the pulsar is now within 10 pc of the galactic plane at a distance of 2 kpc, then it could have travelled in the z direction for no more than 110 pc in its lifetime. The interstellar scintillation properties discussed in ref. 1 suggest a transverse velocity of 110 km s⁻¹ using the correlation diagram of Lyne and Smith⁹. Then the characteristic age is 10^6 yr, and $\Delta P/P \approx 2.5 \times 10^{-3}$. Further discussion of the age of 1937+214 must await analysis of pulse arrival time measurements from a longer interval.

This pulsar may provide us with an extremely stable, classical clock against which we can compare Earth-based atomic clocks. The comparison will require a knowledge of the Earth's orbit about the Solar System barycentre which will exceed the present level of accuracy. According to Mulholland¹⁰ the internal precision quoted above for pulse arrival time fit would make our measurements sensitive to 'believable' errors in the masses of Saturn, Neptune and Pluton in a decade. Observations at multiple frequencies may be necessary to remove variations in dispersion measure.

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Signatures of stellar collapse in electron-type neutrinos

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Neutrinos provide a direct probe for observing the internal convulsions of stars at the end of their thermonuclear life. Implications of developments on the theory of stellar collapse¹ have not been explored systematically for neutrino emission. In view of experiments^{2,3} that can detect extraterrestrial neutrinos, a summary of the theoretical signatures expected in neutrino emission from stellar collapse is needed. If such experiments^{2,3} record an event with features indicative of stellar collapse, its signatures will provide valuable tests for present theories on stellar demise. The current hypothesis⁴ of stellar collapse is that the iron core ($M_c \sim 1.5 M_\odot$) of a massive star ($M_* > 12 M_\odot$) loses its pressure support at the end point of thermonuclear evolution. The core implodes to densities above that of nuclear matter where the collapse halts. The bounce forms a shock at the surface of the core, which then propagates into the infalling mantle. The outcome may be either continued collapse to a black hole or a supernova, depending on the initial mass of the iron core. Here we examine the ν_e emission for both cases. In particular, we give analytical expressions for the rise-time and magnitude of the leading pulse and the average energy of the emitted neutrinos. We then discuss subsequent behaviour of the emission. Ongoing terrestrial experiments can

Table 1 Parameters for PSR 1937+214

Right ascension (1950.0) ¹	19 h 37 min 28.720 s $\pm$ 0.013 s
Declination (1950.0)	21°28'01.3" $\pm$ 0.2"
Dispersion measure	71.20 $\pm$ 0.03 electrons pc cm ⁻³
Period	1 557 806 449 023 fs $\pm$ 110
Apparent period derivative	(1.24 $\pm$ 0.25) $\times$ 10 ⁻¹⁹ s s ⁻¹
Epoch (JED)	2,445,303.263

resolve much of the emission's structure up to distances of ~ 2 kpc.

Our considerations assume a power-law profile for the pre-shock density that was noted⁴ in the results of various hydrodynamic calculations, and use the fact that degenerate relativistic electrons dominate the preshock pressures. Thus the unshocked structure of the infalling envelope is given by

$$\rho_0 = \frac{C}{r^3}; \quad p_0 = K_e p_0^{4/3}; \quad (1)$$

$$\mu_0 = \frac{p_0}{N_0 \rho_0} = \frac{K_e C^{1/3}}{N_0 r}$$

where $C \approx 4 \times 10^{31} \text{ g}$ from hydrodynamic studies⁵, $K_e = 1.23 \times 10^{15} X_e^{4/3} \text{ c.g.s.}$, with X_e being the electron mole fraction per gramme of matter, N_0 is Avogadro's number, and all other notation is standard. From current hydrodynamic studies it is further found⁴ that the preshock velocity profile satisfies the power law

$$u_0^2 = u_{0e}^2 \left(\frac{r_e}{r_s} \right), \quad (2)$$

where the constant $u_{0e}^2 r_e$ is determined from numerical calculations.

Let us first examine the characteristics of the pulse of neutrinos at shock breakout to optical depths below unity. Coherent scattering dominates in the pre-shock envelope, so that the opacity is given by $K_0 \varepsilon_e^2$ where ε_e is the energy of the neutrinos in units of the electron's rest mass $m_e c^2$. Taking iron for the main constituent in the envelope, we obtain $K_0 = 1.0 \times 10^{-20} \text{ cm}^2 \text{ g}^{-1}$. For a thermal distribution the mean neutrino energy is $\bar{\varepsilon}_e = 3fkT_s$, where T_s is the post-shock temperature and f is a factor of the order of unity that increases with the degeneracy of the neutrinos. The mean neutrino energy can be expressed in terms of a shock-strength parameter α as

$$\bar{\varepsilon}_e = 3f\beta \left[\frac{p_s \rho_0}{p_0 \rho_s} \right] \mu \equiv 3f\beta \alpha \mu_0 \quad (3)$$

where β is the ratio of nucleon-to-total pressure and α is defined by equation (3). At shock breakout the neutrino degeneracy parameters are around two, so that $f \sim 1.2$, and typically, $\beta \approx 2/3$ and $X_e \approx .43$. We use these values below unless otherwise stated. During shock breakout, $f\beta\alpha$ should be roughly constant, so that equations (3) and (1) imply $\bar{\varepsilon}_e \propto r_s^{-1}$. With the above assumptions the optical depth in front of the shock is given by

$$\tau_0^s = \int_{r_s}^{\infty} K_0 \bar{\varepsilon}_e^2 \rho_0 dr = \left(\frac{r_0}{r_s} \right)^4 \quad (4)$$

where

$$r_0 = K_0^{1/4} C^{5/12} \left(\frac{3}{2} \frac{f\beta\alpha}{m_e c^2} \frac{K_e}{N_0} \right)^{1/2} = 87 \text{ km} \left(\frac{7\alpha}{25} \right)^{1/2} \quad (5)$$

Note that r_0 is the radius of the shock at breakout ($\tau_0^s = 1$). Likely⁶ values of α are given with $\rho_s/\rho_0 \sim 7$ and $p_s/p_0 \sim 25$. Using the Rankine-Hugoniot relations and assuming that $u_0/D \sim -1$, where u_0 is the pre-shock infall velocity and D is the shock speed, we find that $\alpha \sim 2/3M_s^2$, where M_s is the Mach number of the shock relative to the pre-shock sound speed. Therefore, r_0 is proportional to the Mach number ($\sqrt{\alpha}$) and mildly dependent on the density parameter ($C^{5/12}$).

We now assume that the ν_e s behind the shock attain β -reaction equilibrium⁷ with the matter, so that they have a mole fraction per gramme of matter given by $X_{\nu_e} \approx 0.085$. Taking the emission of ν_e s at the shock front to be isotropic, we find that their number rate of emission as seen by a distant observer, N_{ν_e} , is given by

$$N_{\nu_e} = 4\pi r_s^2 \left(\frac{C}{4} N_0 \rho_s X_{\nu_e} \right) \exp[-\tau_0^s]$$

$$= N_{\nu_e}^0 \left(\frac{r_0}{r_s} \right) \exp \left[- \left(\frac{r_0}{r_s} \right)^4 \right] \quad (6)$$

where

$$N_{\nu_e}^0 = \pi c N_0 \frac{\rho_s}{\rho_0} \frac{X_{\nu_e} C}{r_0} \approx 1.5 \times 10^{59} \text{ s}^{-1} \left(\frac{X_{\nu_e}}{0.085} \right) \left(\frac{25}{7\alpha} \right)^{1/2} \quad (7)$$

and sub(super)scripts s indicate post-shock parameters. Equation (6) assumes an isotropic distribution of neutrinos at the shock front. The exponential represents the beam attenuation factor due to interactions with the unshocked infalling envelope. The peak emission rate occurs for $r_s/r_0 = \sqrt{2}$ with $N_{\nu_e}^{\text{peak}} \approx 8.3 \times 10^{58} \text{ s}^{-1} (25/7\alpha)^{1/2}$. The rise-time for the prompt burst is given by

$$t_r \equiv \left(\frac{N_{\nu_e}}{N_{\nu_e}^0} \right)_{r=r_0} = \frac{r_0}{3D}$$

$$= \frac{K_0^{3/8} C^{11/24}}{2} \left(\frac{f\beta}{N_0 m_e c^2} \right)^{3/4} \left(\frac{K_e \alpha}{6} \right)^{1/4} \quad (8)$$

where the final term on the right expression assumes $\alpha = 2/3M_s^2$. For typical values of the parameters $t_r \approx 0.87 \text{ ms} (7\alpha/25)^{1/4}$, and the rise-time varies only mildly with shock strength.

A primary observable, the average ν_e energy at breakout, is now given by

$$\bar{\varepsilon}_{\nu_e}(\tau_0^s = 1) = \left(6m_e c^2 f\beta\alpha \frac{K_e}{N_0} \right)^{1/2} K_0^{-1/4} C^{-1/12}$$

$$\approx 14.1 \text{ MeV} \left(\frac{7\alpha}{25} \right)^{1/2} \quad (9)$$

Here we see that $\bar{\varepsilon}_{\nu_e}(\tau_0^s = 1)$ is also proportional to M_s .

Because N_{ν_e} is inversely proportional to M_s and $\bar{\varepsilon}_{\nu_e}$ is directly proportional to M_s , the product, the energy luminosity, L_{ν_e} , is

Table 1 Neutrino emission and detector signals

	Prompt emission (ν_e)	Deleptonization (ν_e)	Pair emission		
			(ν_e)	($\bar{\nu}_e$)	($\nu_{\mu,\tau} \bar{\nu}_{\mu,\tau}$)
Δt (ms)	3–10	$\sim 10^3$	$\sim 10^4$	$\sim 10^4$	$\sim 10^4$
$\bar{\varepsilon}_e$ (MeV)	10–20	~ 10	~ 10	~ 10	~ 20
$\Delta t \bar{N}_e$ ($\times 10^{56}$)	2	~ 10	~ 20	~ 20	~ 40
S_{HSK}	7	28	77	1,500	43
$S_{\text{HSK}}/B_{\text{HSK}}$	2	3	4	71	2
S_{IMB}	370	1,500	4,100	8×10^4	2,300
$S_{\text{IMB}}/N_{\text{IMB}}$	17–10	4	4	80	2

A distance of 1 kpc ($3.1 \times 10^{21} \text{ cm}$) to the stellar collapse is assumed. The signal strengths scale with the inverse square of this distance. The duration of each emission phase is denoted by Δt , S is the number of neutrino interactions with the Homestake² (HSK) and Irvine-Michigan-Brookhaven³ (IMB) detectors, B_{HSK} is the background count from the solid curve in Fig. 3 of ref. 2, and $S_{\text{IMB}}/N_{\text{IMB}}$ is based on photomultiplier noise of 2×10^6 counts per s and 4 counts per neutrino event as given in ref. 3. With coincidence counting of signals from individual detector faces and the effects of the forward directionality of electron scattering, the signals-to-noise of the IMB detector can be increased significantly. Note that around a half of the prompt emission occurs between shock breakout and the decay of the photospheric emission with a duration $\lesssim 3 \text{ ms}$. For pair emission, equal energy loss due to each neutrino type is assumed, resulting in lower particle numbers for μ and τ neutrinos having higher particle energies.

independent of the shock strength:

$$L_{\nu_e} = 2\pi N_0 m_e c^3 \frac{\rho_s}{\rho_0} \left(\frac{C}{K_0}\right)^{1/2} X_{\nu_e}^s \left(\frac{r_0}{r_s}\right)^2 \exp\left[-\left(\frac{r_0}{r_s}\right)^4\right] \\ \approx 3.5 \times 10^{54} \text{ erg s}^{-1} \left(\frac{r_0}{r_s}\right)^2 \exp\left[-\left(\frac{r_0}{r_s}\right)^4\right] \quad (10)$$

The peak luminosity of $\sim 1.5 \times 10^{54} \text{ erg s}^{-1}$ is remarkably insensitive of details, depending only on the pre-shock scattering opacity (K_0), the density profile (C), ρ_s/ρ_0 , and $X_{\nu_e}^s$, but not on α or f . However, the initial pulse from stronger shocks will have higher $\bar{\epsilon}_{\nu_e}^{\text{peak}}$ s, lower $N_{\nu_e}^{\text{peak}}$ s and slightly longer rise-times, t_r . Furthermore, all these observables are direct measures of the Mach number.

The above analysis treats the neutrino emission adequately so long as the post-shock matter remains opaque to ν_e s. Note that this is the case at initial shock breakout as the neutrino mean-free path decreases across the shock. The dominant factor behind the decrease is the density contrast across the shock of around a factor of seven. The opacity, however, decreases only slightly across the transition. From ref. 7, in the discussion following their equation (8), one has for the opacity behind the shock, $\frac{3}{4} N_0 \sigma_0 \epsilon_{\nu_e}^2$, where $\sigma_0 = 1.7 \times 10^{-44} \text{ cm}^2$, and for the opacity ahead of the shock (due to coherent scattering off 'iron'), $N_0 \sigma_0 \epsilon_{\nu_e}^2$.

As the shock reaches lower densities, the post-shock matter will become increasingly transparent. At some stage, the opaque region will be left behind by the shock. We denote the transition region between the transparent and opaque matter by the term 'neutrinosphere'. Hence, the situation will evolve where the shock leaves a transparent region behind that electron captures until the absorption of neutrinos from the interior neutrinosphere can balance the electron capture. When the balance is achieved the matter can contribute further to the neutrino emission only to the extent that the neutrinospheric emission decreases. At this point there will be two major components of the neutrino emission: the first being the emission from the neutrinosphere; the second the emission from a thin shell behind the shock. This is due to the rapid electron capture on protons just behind the shock in the entering material that continues until this matter attains kinetic equilibrium with the neutrinospheric emission⁷.

For the typical profiles obtained in hydrodynamic calculations, the neutrinospheric emission decays exponentially^{7,8}. The shell emission behind the shock depends on the rate of matter flow across the shock and the decrease in electron fraction required to attain kinetic equilibrium⁷. At this stage, our considerations must ramify to consider the two possibilities: supernova explosion with mass ejection, or shock stagnation with continued accretion. We proceed by making the simplifying assumptions that D is constant for a supernova, while $D = 0$ for shock stagnation. This should adequately represent the shock behaviours for our purposes because most of the neutrino emission will occur while the shock traverses the regions of rapid electron capture with post-shock densities of 10^{11} and $10^{10} \text{ g cm}^{-3}$. For the case of successful shocks, this range should be sufficiently narrow so that the shock speed will not change appreciably. On the other hand, unsuccessful shocks do stagnate in just this density range.

For the supernova case with constant D , one has $r_s - r_e = Dt$, where t is measured from shock breakout and the subscript e refers to the neutrinosphere. In addition one has equation (2), where now it is seen that μ_{0e} denotes the pre-shock velocity and r_e the radius of the emission surface. With these assumptions and published⁷ expressions for the neutrinospheric and electron capture emission we obtain the following total neutrino emission after breakout

$$N_{\nu_e} = N_{\nu_e}^0 \left(\frac{r_0}{r_e}\right) \left\{ e^{-t/\tau_e} + \frac{\gamma}{1 + Dt/r_e} \left[1 + \frac{|\mu_{0e}|/D}{(1 + Dt/r_e)^{1/2}} \right] \right\} \quad (11)$$

where $\gamma = (4D/c)(\Delta X_e/X_{\nu_e}^0)(\rho_0/\rho_s)_e$, ΔX_e is the difference

between pre-shock and kinetic equilibrium electron fractions, $X_{\nu_e}^0$ is the initial value of the neutrino fraction at the point where the neutrinosphere first becomes static, and the neutrinospheric decay time is given⁷ by $\tau_e \approx 4.5 r_e/c \approx 1.5 \text{ ms}$ ($r_e/100 \text{ km}$).

For typical^{5,7,8} parameters of $D \sim 3 \times 10^9 \text{ cm s}^{-1}$ and $\Delta X_e/X_{\nu_e}^0 \sim 3$, $\gamma \sim 0.17$. As $|\mu_{0e}|/D \sim 1$ initially, the contribution of the shell term (the second term in the curly brackets) to the total initial flux can be sizeable ($\sim 1/4$). This shell source, which does depend linearly on the shock speed, should be incorporated into our estimate of the peak number and energy fluxes at breakout. Reasonable assumptions now yield for $N_{\nu_e}^{\text{peak}}$ a value of $\sim 1.1 \times 10^{59} \nu_e \text{ s}^{-1}$ and for $L_{\nu_e}^{\text{peak}}$ a value of $\sim 1.8 \times 10^{54} \text{ erg s}^{-1}$, where $L_{\nu_e}^{\text{peak}}$ now has a soft dependence on the shock strength and the Mach number because of the shell component. As τ_e is only $\sim 1.5 \text{ ms}$, the neutrinospheric flux decays away quickly. Hence, in 2–3 ms, the shell component takes over as the prime source of ν_e s. As the shock traverses the region of rapid electron capture, outside of the neutrinosphere, between post-shock densities of 10^{11} and $10^{10} \text{ g cm}^{-3}$, $\bar{\epsilon}_{\nu_e}^{\text{cap}} \sim 10 \text{ MeV}$, so that the ν_e spectrum softens with time relative to the energy of the prompt burst in equation (9).

Emission in the failed shock scenario is obtained similarly. In this case, the general evolution of the ν_e flux is similar to that in the successful case until the shock starts to stall and stagnates ($D = 0$). This happens $\sim 5 \text{ ms}$ after breakout⁸. At this stage, the neutrinospheric flux is negligible, and, if pre-shock conditions were to remain constant, the second term in the curly brackets of equation (11) would give a constant rate emission. However, the power laws of equations (1) and (2) dictate changing pre-shock conditions. With these relations the fluid equation for mass continuity gives

$$\frac{1}{\rho_0} \frac{\partial \rho_0}{\partial t} = + \frac{3}{2} \frac{u_0}{r_s} = - \frac{3}{2} \frac{|\mu_{0e}|}{r_e} \left(\frac{r_e}{r_{\text{stg}}}\right)^{3/2} \quad (12)$$

Assuming that the right-most expression is roughly constant, one can integrate to obtain an exponential decay for the pre-shock density with a time constant of

$$\tau_{\text{stg}} \approx \frac{2}{3} \frac{r_e}{|\mu_{0e}|} \left(\frac{r_{\text{stg}}}{r_e}\right)^{3/2} \quad (13)$$

For typical values^{5,8} of $|\mu_{0e}| \sim 3 \times 10^9 \text{ cm s}^{-1}$, $r_e \sim 80 \text{ km}$, and $r_{\text{stg}} \sim 200 \text{ km}$, This decay time is $\sim 6.3 \text{ ms}$. Once the shock stalls, the neutrino emission is

$$N_{\nu_e}^{\text{stg}} = \gamma_{\text{stg}} \left(\frac{r_0}{r_e}\right) N_{\nu_e}^0 e^{-t/\tau_{\text{stg}}},$$

with

$$\gamma_{\text{stg}} = \left(\frac{\rho_0}{\rho_s}\right)_e \frac{4|\mu_{0e}|}{c} \frac{\Delta X_e}{X_{\nu_e}^0} \left(\frac{r_e}{r_{\text{stg}}}\right)^{3/2} \quad (14)$$

The above parameters yield $\gamma_{\text{stg}} \approx 0.07$ so that $N_{\nu_e}^{\text{stg}} \sim 10^{58} \exp[-t/\tau_{\text{stg}}] \text{ s}^{-1}$ after stagnation. Note that both the 'stagnant' electron capture and neutrinospheric emissions decay exponentially, but with dramatically different time constants.

In the case of both supernova and failed shock, one expects a continuous decrease in the emission from electron capture in newly accreted matter. However, the total emission cannot decrease below a minimum value. This minimum value of the emission rate is due to the diffusion of trapped neutrinos from the central regions. The central core which contains a large number of trapped leptons will be losing them by neutrino diffusion on time scales of several hundreds of milliseconds⁹. This deleptonization flux starts to assert itself $\sim 10 \text{ ms}$ after breakout when its value is $\sim 2 \times 10^{57} \text{ s}^{-1}$.

The salient features of neutrino emission during stellar collapse can be summarized as follows. Shock breakout gives a

prompt burst with a rise-time of ~ 1 ms, followed by an ~ 1 ms neutrinospheric decay. After a few milliseconds, the electron capture flux starts to dominate (if the shock stalls, it stalls about now) and continues to dominate for 20–30 ms after which time the long-term core deleptonization flux takes over. The relevant flux decay time constant changes from 1 to ~ 10 ms to hundreds of milliseconds as each of the respective phases assumes importance. Hydrodynamic results⁴ show that $\bar{\epsilon}_{\nu_e}$ decreases by about one-third from its maximum value at shock breakout. The main characteristics of neutrino emission are given in Table 1. For completeness, we include the long-term emission in pairs of particle–antiparticle neutrinos. Essentially all the energy of formation of a final cold neutron star ($\sim 2 \times 10^{53}$ erg) is carried away by this pair emission, and the latter is prominent in discussions^{2,3} of detectability.

Detection by neutrino–electron scattering¹⁰ can occur for all of the neutrino types. Detection by absorption of neutrinos is possible for only $\bar{\nu}_e$ and proton reactions¹¹ in water detectors. For equal mixes of neutrino types, detection by absorption overwhelmingly dominates (>20 absorptions for each scatter of particles at energies ≥ 10 MeV). However, due to the slow rate of pair production relative to electron capture, the initial burst has essentially a pure ν_e composition on time scales ≤ 10 ms. The $\bar{\nu}_e$ emission is likely to become significant after several tens of milliseconds.

The signal from a stellar collapse at 1 kpc is shown in Table 1, together with signal-to-background ratios (signals scale with the inverse square of the distance). The prompt neutrinos are detectable (signal-to-background ≥ 2) up to distances of 1 and 3 kpc in the Homestake² (HSK) and the Irvine–Michigan–Brookhaven³ (IMB) experiments, respectively. Both experiments would see the remaining structure only at distances below 2 kpc. The signal strength in the pair $\bar{\nu}_e$ component is striking, where both detectors are sensitive out to distances of ~ 7 kpc. With coincidence counting of $\bar{\nu}_e$, range of IMB detector can be extended to include the entire Galaxy.

A complication not treated in our considerations is the effect of pulsations. The dominant ν_e emission is from densities between $\sim 10^{11}$ and 10^{10} g cm⁻³. Matter at these high densities should not be ejected even in the supernova case. Its recollapse leads to core and mantle pulsations. The pulsations give density variations which appreciably affect the rate of electron capture. We estimate that such pulsations can give a periodic modulation of the basic neutrino emission discussed above, with an amplitude between 25 and 50% of the total electron capture emission and periods between 10 and 20 ms.

Distinguishing ν_e signals from supernova and stalled shock events may be difficult. Our considerations above indicate that the prompt ν_e burst will not differ markedly for the two cases. The fact that matter recollapses in both cases indicates similar ν_e emissions on longer time scales of tens of milliseconds. However, the secondary pulse resulting from recollapse should occur sooner for the stalled shock case, and somewhat later for a supernova. The time separating the prompt and secondary burst should correspond to the pulsation times of ~ 10 –20 ms.

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A new AM Her-like X-ray source

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Among low luminosity galactic X-ray sources, AM Herculis-type binaries form a distinct class. The main characteristics of this group of binaries are: orbital periods of the order of a few hours, the presence of emission lines of H I, He I, He II, C III and N III, variable linear and circular polarization of the optical radiation, and emission of a strong and variable soft X-ray flux. To date six such binaries have been detected. Four of these—AM Her^{1,2}, AN Ursae Majoris^{3,4}, 2A0311–227^{5,6} and VV Puppi^{7,8}—have been known for some time, while E1405–451⁹ and a new serendipitous source near NGC3607¹⁰ were detected only recently from the Einstein satellite observations. It is generally believed that these binaries consist of a magnetic white dwarf primary star and a red dwarf companion star¹¹. The X-ray emission then arises due to accretion of matter from the red dwarf secondary onto the poles of the magnetic white dwarf¹². We now report the discovery of a new X-ray source and its optical identification with a binary which has the necessary X-ray and optical characteristics to qualify it as a new member of the AM Her class of objects.

The X-ray observations were carried out with the low energy detector 1 (LED1) of the HEAO A-2 experiment¹³ and the Imaging Proportional Counter (IPC) on the Einstein Observatory¹⁴. From superposed scans of LED1 for the days 15–23 November 1977 a new X-ray source, now designated H0139–68, was discovered from the plot of count rate versus scan angle for the 0.15–0.4 keV band. The position of the new source was confirmed during its transit over the observatory 6 months later in the summed scans of 13–18 May 1978. The source was detected only in the 0.15–0.4 keV band indicating a very soft source spectrum. Analysis of individual source scans showed its intensity to be highly variable from scan to scan. Intensity, corrected for offset from the LED1 axis, is plotted in Fig. 1 as a function of time for the two transits of the source. Intensity variations of a factor of two or more over time scale as short as ~ 2 h can be clearly seen. Although HEAO-1 scans a given source once every 33 min, we have useful data for only limited number of scans due to data drop out, Earth occultations and various other factors. As a result we are unable to discern any periodicity which might be present in the data.

An error box for the source was derived by overlapping 2.95° FWHM field of view (FOV) of LED1 perpendicular to the scan direction, for the first and last sightings of the source for which a definite detection was made. Figure 2 shows the positions of LED1 FOV corresponding to the first and last source sightings by continuous lines. The source should be within the overlapping region shown by dashed lines in Fig. 2. This is therefore the error box for the source which has an area of 0.9 deg². The size of the error box along the scan direction was derived from uncertainty, at the 90% confidence level, in the determination of the scan angle from the summed scan data. Also shown in Fig. 2 is the error box of the Ariel V source 3A0142–681¹⁵ which we suggest is the same source as H0139–68 (see below). Figure 3 shows an enlarged section of the error box, indicated in the dotted rectangle in Fig. 2, superposed on an ESO print for that part of the sky. To obtain a more precise position of the X-ray source in order to allow its optical identification, IPC observations of three fields covering the HEAO A-2 error box were carried out under the guest investigation programme of the US National Aeronautics and Space Administration. No source was detected in two of the three

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Fig. 1 Count rate for H0139-68 shown for different source scans made in 1977 and 1978 with the LED1.

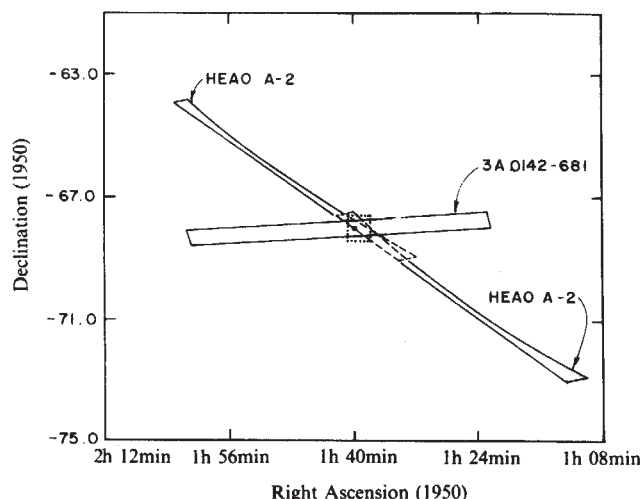
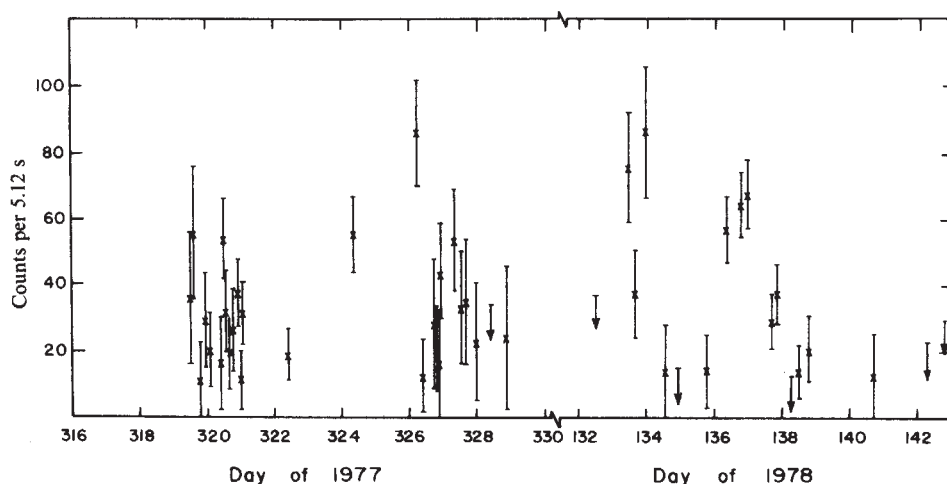


Fig. 2 Positions of the 2.95° fields of view of the LED1 of the HEAO A-2 experiment are shown by continuous lines for the first and last source sightings. The error box of H0139-68 is indicated by the dashed lines. An enlargement of the portion of the error box within the dotted rectangle is shown in Fig. 3. The error box of the Ariel V source 3A0142-681 thought to be identical with H0139-68 is also shown here.

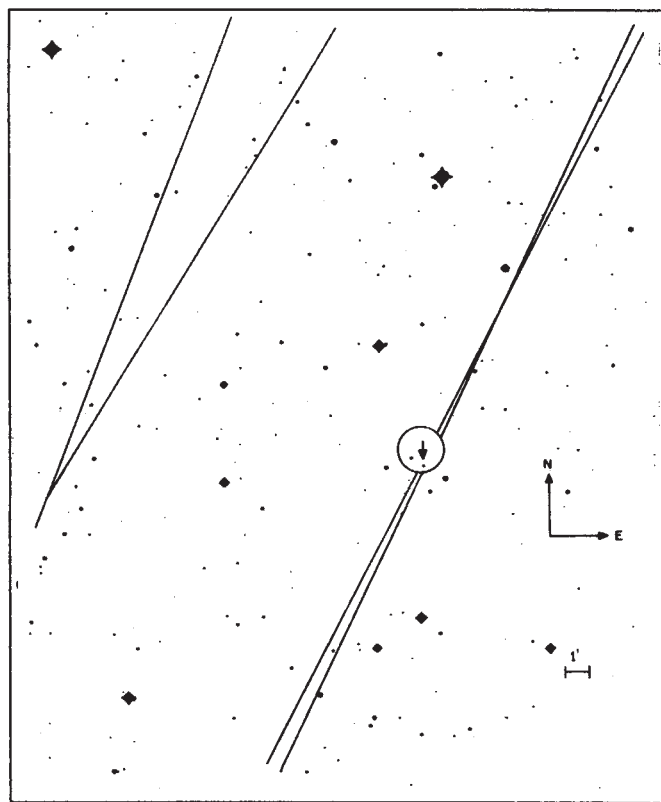


Fig. 3 Part of LED1 error box shown by the dotted rectangle in Fig. 2, shown by continuous lines superposed on an ESO sky print for that region of the sky. The IPC error circle of 1 arc min diameter is also shown, and the optical counterpart of H0139-68 is indicated by an arrow in the IPC error circle.

IPC fields. Observations of the third field carried out on 20 November 1979 between 14:43 and 15:15 UT revealed the presence of a bright soft X-ray source. The source was observed for a total duration of 1,836 s which, correcting for vignetting and other instrumental effects, corresponds to an effective exposure time of 1,582 s. The best-fitting source position derived from the IPC observations is RA (1950) = 01 h 39 min 36 s, dec (1950) = $-68^\circ 07' 57''$. Based on count rate statistics alone, the error circle radius at 99% confidence level is estimated to be $16''$. However, systematic uncertainties like those in the aspect determination are much larger and therefore the true uncertainty is more likely to be about 1 arc min (F. Seward, personal communication). An error circle of 1 arc min radius centred on the best fit source position superposed on the ESO print is shown in Fig. 3. Two faint stars are detected within the error circle. Based on the rapid variability and soft nature of X-ray emission as well as faintness of the optical candidates, we suspected that H0139-68 is most likely an AM Her-type source. We therefore sent identification chart for the two candidate stars to N. Visvanathan (Mount Stromlo and Siding Spring Observatory) who made photometric and spectroscopic observations of the stars. The star shown by an arrow in the IPC circle has been identified as the optical counterpart of H0139-68 from optical observations by Visvanathan and Pickles¹⁶. The second star within the error circle is an ordinary late type star with no indication of variability.

Strong support for identifying the object as AM Her-like is provided by the optical observations¹⁶. Radial velocity as well as photometric measurements show that the optical counterpart of H0139-68 is a binary with an orbital period of 113.65 min (N. Visvanathan, personal communication). This is confirmed by the photometric observations of Cropper¹⁷. The magnitude of the object varies between 14.9 and 16.4. Flickering, a common characteristic of AM Her-like system, is seen throughout the optical light curve over time scale of minutes. The AM Her-like classification of H0139-68 is also supported by the observed steep rise of the optical flux to maximum seen after the primary eclipse^{16,17}. Strong evidence for the presence of a magnetic white dwarf in the binary is provided by the linear polarization measurements of Visvanathan *et al.*¹⁸ who detected a polarization spike of 7% amplitude in the polarization phase curve. This suggests that a part of the optical continuum is due

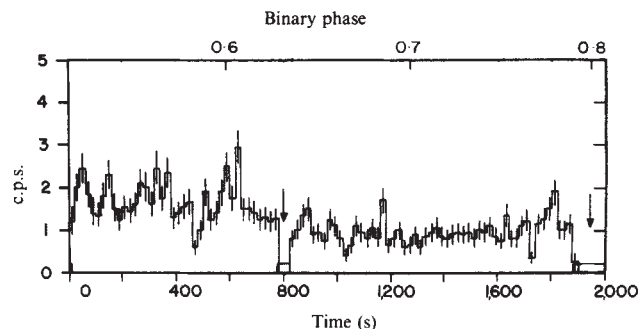


Fig. 4 Observed IPC counting rate for H0139-68 plotted as a function of time. Time $T = 0$ s corresponds to 14 h 43.5 min UT on 20 November 1979.

to cyclotron emission originating at poles of the magnetic white dwarf.

A total of 2,364 counts, corresponding to an average count rate of 1.3 c.p.s., were detected from the IPC cells within a 2 arc min radius of the source position. This includes a contribution of 19 background counts. Time analysis of count rate showed intensity to be variable over time scale of 20 s and more by a factor of up to 3. Figure 4 shows a plot of count rate against time, corrected for exposure efficiency, in 20-s time bins. Several features can be seen in Fig. 4: (1) The average count rate dropped by a factor of about 2 after $T + 900$ s where T is the time at the beginning of observation; (2) up to $T + 900$ s when the source is in a bright state, the count rate varies over time scale of 50–100 s by a factor of about 2; (3) flickering on time scale of ~ 20 s. Erratic intensity variations and flickering observed for H0139-68 are similar to those reported for AM Her¹⁹. Although there is suggestion of cyclic variations in intensity in the bright phase, no coherent intensity oscillations are present throughout the data. Similar quasi-cyclic intensity variations are discernible in the X-ray light curve of AM Her reported by Fabbiano *et al.*²⁰. More detailed timing analysis of the H0139-68 data is in progress.

The energy spectrum of the source measured with the LED1 as well as IPC is found to be very soft. The spectrum for three LED1 scans of 16 May 1981 when the source was in a bright state, is well described by an exponential spectrum (including the gaunt factor) with $kT \leq 0.09$ keV and an interstellar hydrogen column density (N_H) $\leq 3 \times 10^{21}$ cm⁻². Due to rather poor energy resolution of the IPC as well as variation of its gain with position and time, the spectral information derived from it is subject to considerable uncertainty. Nevertheless we attempted to fit the observed IPC count spectrum shown in Fig. 5 with the usual spectral models. No acceptable fits were found for any of the attempted models. The exponential spectrum gave kT in the range of 0.01–0.1 keV for $N_H \leq 10^{21}$ H cm⁻² while corresponding range of kT was 0.01–0.05 keV and $N_H \leq 4 \times 10^{20}$ cm⁻² for the black body spectrum. In Fig. 5 a black body spectrum fit with $kT = 0.03$ keV and $N_H = 4 \times 10^{20}$ H cm⁻² is shown as a histogram. The soft nature of the spectrum of H0139-68 shows marked resemblance to that of AM Her²¹.

Mchardy *et al.*¹⁵ have recently published the 3A catalogue of X-ray sources at high galactic latitude using Ariel V satellite observations in the 2–18 keV band. The error box of one of the sources 3A0142-681 listed in this catalogue overlaps with the error box for H0139-68 as seen in Fig. 2. The optical counterpart of H0139-68 lies within the error box of 3A0142-681 which strongly suggests that this source is the same as H0139-68. If this is true, then in that case H0139-68 has a hard spectral component in X-ray emission which probably has an origin different from the soft component observed by us. The presence of a hard X-ray component (≥ 2 keV) provides further support for AM Her-like nature of H0139-68 since AM Her and 2A0311-227, both have hard X-ray components^{22,23}.

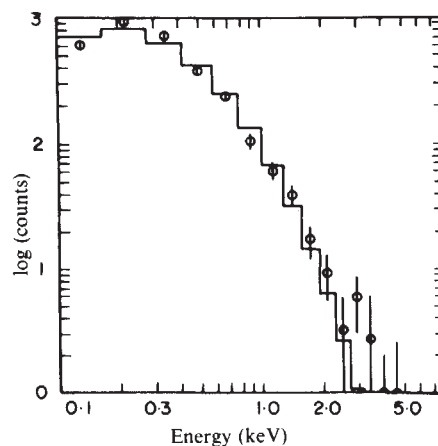


Fig. 5 A plot of counts against energy as observed with the IPC. The histogram is a black body spectrum fit with $kT = 0.03$ keV and $N_H = 4 \times 10^{20}$ H cm⁻², to the observed data points.

Using the black body spectrum with $kT = 0.03$ keV and $N_H = 4 \times 10^{20}$ H cm⁻², we estimate the source flux to be 2.3×10^{-10} erg cm⁻² s⁻¹ which corresponds to X-ray luminosity (L_X) of 2.7×10^{32} erg s⁻¹ for a distance of 100 pc, which is usually assumed for the nearest cataclysmic variables. The hard X-ray flux determined from the Ariel V count rate, corresponds to 1.05×10^{-11} erg cm⁻² s⁻¹ in 2–10 keV band and gives $L_X \approx 1.2 \times 10^{31}$ erg s⁻¹ which indicates that $(L_X)_{\text{soft}} > (L_X)_{\text{hard}}$. Patterson²⁴ has recently pointed out that the ratio L_X/L_{opt} is a good indicator for distinguishing a white dwarf from a neutron star in binary sources. He found $L_X/L_{\text{opt}} \sim 10^3$ for neutron stars and ~ 1 for the white dwarfs. Using the flux value of Mchardy *et al.*¹⁵ and $m_v = 14.9$ we estimate $L_X/L_{\text{opt}} \sim 3$ in agreement with the white dwarf nature of the accreting object in H0139-68.

The hard X-ray flux from H0139-68 could represent the bremsstrahlung component from the hot emission region of the white dwarf as suggested by the calculations of Lamb and Masters²⁵ for AM Her-type binaries. The intense soft X-ray flux could then be explained as the black body emission from the polar surface of the white dwarf which is heated by the bremsstrahlung X-ray and UV cyclotron emission components. This is believed to be the case for AM Her. If such an explanation is tenable, then H0139-68 and other AM Her-like objects should be bright UV sources. UV observations of AM Her with the International Ultraviolet Explorer (IUE), however, do not show the predicted strong UV flux²⁶ which makes it difficult to explain the origin of the soft X-ray flux in these sources. IUE observations in the UV band and further X-ray observations covering the entire binary phase of H0139-68 are required for a better understanding of the nature of this new binary.

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Chemical amplification of optoacoustic signals

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Historically, the optoacoustic effect has been produced in gases through the absorption of IR radiation¹⁻³. As the effect depends only on the conversion of modulated radiation to periodic heating of the gas (which in a closed vessel is equivalent to a sound wave), it is not surprising that the optoacoustic effect can be produced by several different interactions where radiation is absorbed by matter. Here we report generation of the optoacoustic effect by photochemical release of energy through a chain reaction mechanism in H₂-Cl₂ mixtures. A unique feature of the optoacoustic effect produced in this manner is chemical amplification of the acoustic signal caused by a release of energy from the chemical reactions that is greater than the energy absorbed from the light beam. In addition, because the acoustic signal depends on the kinetics of the reaction and the time dependent concentrations of the reactants, the optoacoustic effect acts to monitor directly the progress of the reaction in time.

The apparatus for the experiments, as shown in Fig. 1, consisted of a 1.3-cm diameter spectrophone cell, 7.6-cm long with Pyrex windows at either end. A modified condenser microphone (Bruel and Kjaer Inc., Model 4125) was mounted in a small chamber adjacent to the spectrophone cell. To prevent chemical attack of the preamplifier (which is usually attached directly to the microphone) a short lead was connected to the backing plate of the microphone and fed through the cell wall to a high

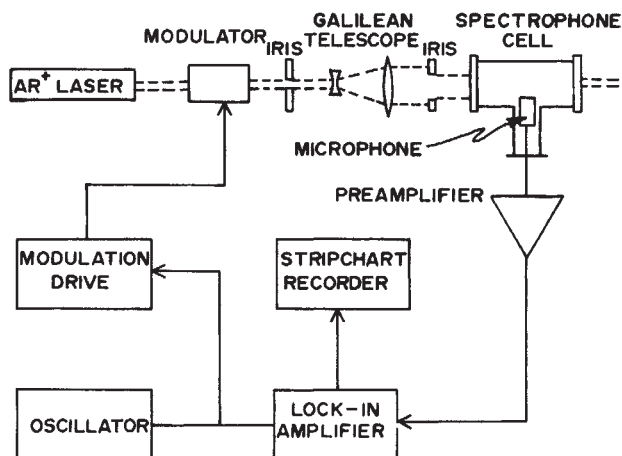


Fig. 1 Schematic diagram of the experimental apparatus used to record optoacoustic signals in a static mixture of H₂ and Cl₂ in a buffer of N₂.

input impedance external preamplifier. (Due to the corrosive nature of the gases, the diaphragm on the microphone was replaced by 1.3 × 10⁻²-cm thick Ni foil that was welded to the housing. The membrane and weld were then coated with Hypalon, chlorosulphonated polyethylene paint.) The radiation source for the experiments was an Ar ion laser operated on the 476.5-nm line. The laser beam was amplitude modulated at a frequency of 20 Hz by an acousto-optic light modulator and expanded in a galilean telescope so that the light filled the spectrophone volume with an approximately constant intensity profile. Signals from the microphone were detected by a lock-in amplifier with the phase adjusted for maximum amplitude.

A mixture of H₂ (20%), Cl₂ (70%) and N₂ (10%) was passed through the spectrophone cell. After 15 min the gas flow was stopped, the laser beam was directed into the cell and a recording of the acoustic signal was made. Between each measurement the above procedure was repeated to ensure that all reaction products were flushed from the cell. The light modulator was adjusted to give a modulation depth of 0.1; that is, 10% of the light intensity was modulated as sin ωt while 90% of the beam remained constant.

The experimentally observed optoacoustic signals, shown in Fig. 2, display a rapid increase in amplitude that falls off in time at various rates depending on the intensity of the laser radiation. That is, as the laser power is decreased (with the modulation depth fixed) the rate of decay of the signal in time also decreases. For laser powers in excess of those given in Fig. 2, explosions occasionally took place. The insert in Fig. 2 shows the time-dependent optoacoustic signal observed in a mixture of Cl₂ (85%) in N₂ (15%). Even with high laser powers only small, relatively noisy acoustic signals were found. The constant amplitude of these signals contrasts greatly with those where H₂ was present.

The optoacoustic effect in pure gaseous Cl₂ takes place primarily by equilibration of the translational recoil energy of the nascent photofragments and eventually through release of the Cl₂ bond energy when the Cl radicals recombine. For the

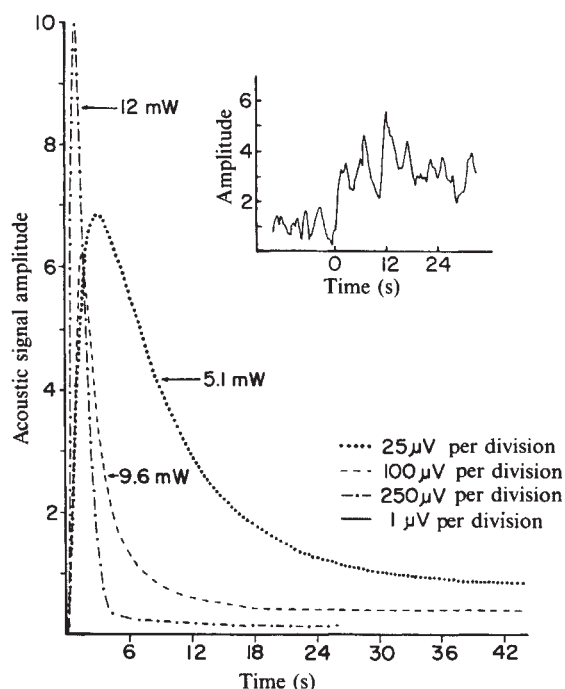


Fig. 2 Optoacoustic signal versus time for mixtures of H₂ + Cl₂ in a buffer gas of N₂ for several laser powers. The rise-time of the signal is determined by the time constant (1 s) set on the lock-in amplifier. The magnitude of the signal after the initial increase depends on the instantaneous value of the reaction rate. The inset shows an optoacoustic signal in a Cl₂-N₂ mixture taken at a power of 75 mW with the lock-in amplifier at high gain (1 μV per division) and a time constant of 3 s.

case at hand, the recoil energy is 20% of the bond energy so that the latter mechanism is the dominant energy release pathway. When H_2 is present, however, the following sequence of reactions takes place,



where $h\nu$ represents the incident radiation and M is a third body (either H_2 , Cl_2 or N_2) that carries away excess energy in the three-body recombination. A well known property of these reactions is that consumption of a Cl radical in reaction (2) is followed by regeneration of another Cl atom through reaction (3). Because k_3 is large, the chain cycle of reaction (2) followed immediately by reaction (3) can be considered instantaneous on the time scale of these experiments so that large amounts of HCl are generated by the introduction of only a small concentration of Cl . As a single Cl radical initiates many chain cycles before recombination (through reaction (4)) the energy release from the chain reactions is much greater than that absorbed from the light beam thereby giving rise to an amplification of the acoustic signal.

A detailed analysis of the optoacoustic effect produced by reactions (1)–(4) has been given by Diebold and Hayden^{4,5} for radiation with a time dependence of the form $\rho B(1 + d \sin \omega t)$, where ρ is the radiation density of the incident light, B is the Einstein coefficient for absorption and dissociation of Cl_2 , d is the modulation depth of the radiation, ω is the modulation frequency, and t is the time. For the light intensities, modulation frequency and concentrations of reactants in these experiments the optoacoustic signal amplitude is found to be

$$p(t) = -\left(\frac{2}{3\omega^2}\right)k_2[H_2][Cl_2]\Delta U_{rx}\rho B d \sin \omega t \quad (5)$$

where ΔU_{rx} is the energy release for the sum of reactions (2) and (3). As reaction proceeds, $[H_2]$ and $[Cl_2]$ decrease in time as a result of the steady component of the radiation. At high incident laser power the photochemical reaction proceeds rapidly, depleting the concentrations of H_2 and Cl_2 ; when the laser power is lowered the reaction rate is reduced correspondingly. Thus, according to equation (5) the optoacoustic signal acts to monitor the instantaneous concentrations of the reactants as the reaction takes place with a signal amplitude proportional to the photodissociation rate and the chain cycle exothermicity.

It is evident that spectrophone measurements can be extended to monitor other exothermic systems as well. The detection of the photolysis products of CH_3NO_2 in a spectrophone cell by Colles *et al.*⁶ as the photolysis was proceeding suggests that both product formation and reaction exothermicity might simultaneously be measured by modulation of two different radiation sources at different frequencies with phase sensitive detection at two frequencies.

The optoacoustic effect produced by photodissociation can be looked on as the frequency domain analogue of flash photolysis; that is, instead of probing the system with a short pulse of light and observing time dependent effects, the optoacoustic method relies on changes that take place when the frequency of modulation of a steady source is varied. Although the methods of detecting changes in the system are different for the time and frequency domain experiments, the information obtained, in general, is identical. A subtle difference between the time and frequency domain experiments is that the former is usually carried out with very intense light sources (to ensure a high signal-to-noise ratio), whereas the latter employs weak sources. Thus the effects of impurities on reaction rates, which

can be expected to be most pronounced at low photodissociation rates, can perhaps be studied with some advantage by sensitive acoustic methods.

There is also a notable similarity between the optoacoustic effect and the rotating sector, or intermittent activation technique⁷. In essence, both techniques rely on measuring changes in overall reaction rates that vary with modulation frequency. The salient feature of the optoacoustic technique, however, is that changes are seen instantaneously and at the same time with remarkable sensitivity.

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Plessite formation by discontinuous precipitation reaction from γ -Fe,Ni in Richardton (H5) ordinary chondrite

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Discontinuous precipitation reactions involve the growth of stable phases behind a grain boundary migrating into a supersaturated solid-solution¹, and have been synthesized in Fe,Ni alloys at temperatures $\geq 500^\circ\text{C}$ (ref. 2). Axon and Grokhovsky³ have recently described localized, low temperature (350 – 400°C) discontinuous precipitation reactions of the type $\alpha \rightarrow [\alpha + \gamma]$ (ref. 2) occurring naturally in initially slowly cooled α -Fe,Ni (kamacite) in the Richardton H-group ordinary chondrite. In this further study of the same meteorite we describe a complementary discontinuous precipitation reaction in zoned γ -Fe,Ni (taenite) which has produced plessite ($\alpha + \gamma$ -Fe,Ni). Microscopic discontinuous precipitation reactions in Richardton metal occurred at lower temperatures (350°C) and, in the case of zoned γ -taenite, at higher Ni contents than those synthesized in the laboratory². We suggest that discontinuous precipitation may be an important mechanism for the production of plessite in strained then annealed, or shock reheated, meteorites.

Solid-state precipitation processes recognized so far in meteoritic Fe,Ni metal mainly involve the growth of a new phase by means of a 'volume' diffusion flux perpendicular to the growing interface. The best known example is the development of the Widmanstätten structure in the octahedrite classes of iron meteorite by the growth of b.c.c. α -kamacite from f.c.c. γ -taenite during slow (1 – $200^\circ\text{C Myr}^{-1}$), equilibrium cooling in the temperature interval 700 – 400°C on their parent bodies^{4–6}.

Although metal in chondritic meteorites is mixed with silicate and sulphide minerals, Wood⁷ has noted that the $\gamma \rightarrow \alpha + \gamma$ process was essentially the same in both chondritic and iron

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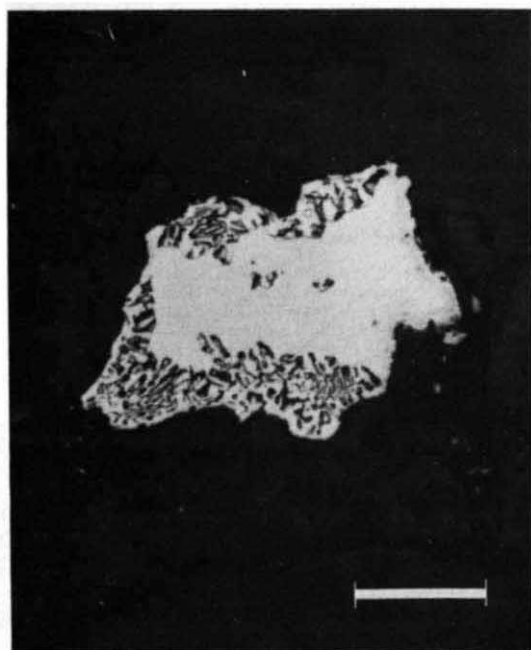


Fig. 1 Zoned γ -taenite grain displaying partial, discontinuous transformation to plessite ($\alpha + \gamma$) along metal-silicate grain boundary (2% nital etch). Scale bar, 50 μm .

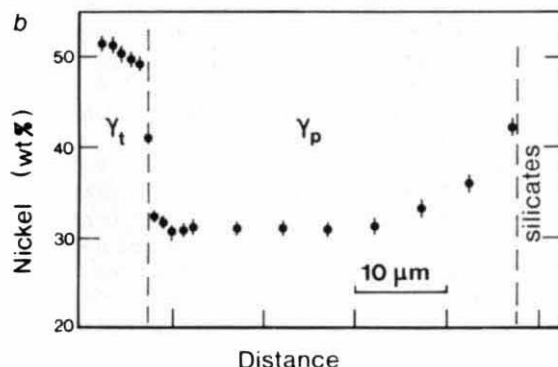
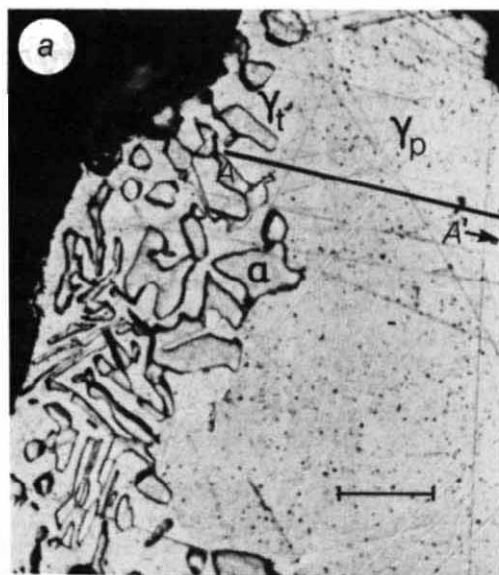


Fig. 2 *a*, Small γ -taenite grain with discontinuous plessitic reaction products (2% nital etch). Scale bar, 10 μm . *AA'* shows the line of the microprobe trace given in *b*.

meteorites. In chondritic materials, in addition to 'volume' diffusion, long-range migration of Ni between metal particles physically separated by silicates may have taken place by 'grain boundary' or 'surface' diffusion.

'Volume' diffusion through crystal structures is slower than either 'grain boundary' or 'surface' diffusion, and volume effects may become 'frozen-in' with falling temperature. In well-annealed, slowly-cooled meteorites this has resulted in residual γ -taenite which is often strongly zoned (20–45 wt%) with respect to Ni.

During cooling, the cores of large, residual, zoned γ -taenite which have retained sufficiently low Ni (20–25 wt%) may undergo a Widmanstätten decomposition to microscopic precipitates of $\alpha + \gamma$ -Fe,Ni called plessite^{6,8}. It has been suggested^{6,8,9} that plessite may also be produced by the isothermal decomposition of shear-related martensitic transformations (α_2) during cooling by the reaction $\gamma \rightarrow \alpha_2 \rightarrow \alpha + \gamma$. The controlling factors for the production of plessite by these mechanisms are composition and cooling rate.

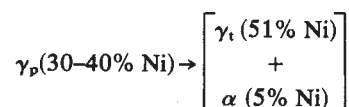
Wood⁷ has shown that most zoned γ -taenite grains in slowly cooled (1–10 °C Myr⁻¹) ordinary chondrites are generally too small to have retained core Ni compositions low enough to allow decomposition to plessite on cooling. However, he suggested that, in some chondrites, mild reheating to temperatures (400–500 °C) still within the two-phase ($\alpha + \gamma$) field of the Fe–Ni system has caused unstable martensite with ~30 wt% Ni to decompose to plessite. Experimental evidence for complete decomposition by the reaction $\gamma \rightarrow \alpha_2 \rightarrow \alpha + \gamma$ has been provided on quenched alloys with 10–20% Ni re-heated to 550 °C for some months¹⁰.

Solid-state reactions at low temperatures, whether during cooling or reheating, depend on atomic migration along paths of especially high diffusivity. Precipitation-induced grain boundary migration in the solid is one mechanism which can provide rapid diffusion paths within, and parallel to a moving interface. In synthetic alloys, transformations of this kind have been termed 'discontinuous precipitation reactions' and involve the formation of two (or more) stable phases behind a grain boundary which is migrating into a supersaturated solid-solution^{1,2}. To date, little consideration has been given to the possibility of discontinuous precipitation as a mechanism for the production of structures in meteoritic metal.

Microscopically, zoned γ -taenite in Richardton occurs contiguously with massive α -kamacite and troilite (FeS) and as apparently isolated grains (10–100 μm) within chondrules, and also in the matrix between chondrules.

Figure 1 shows a grain of 'matrix' γ -taenite which has partially transformed to plessitic plessite. The structure comprises small (1–3 μm), irregular and rod-like α -kamacite precipitates which have grown from points near the metal-silicate boundary of a previously zoned (~30–40 wt% Ni) γ -taenite grain. A 'washed out' remnant of the dark-etching zone¹¹ is seen along the edge which lacks α -kamacite precipitates (Fig. 1 right-hand edge). Most α -kamacite rods do not extend to the metal-silicate boundary but appear to terminate a few micrometres in, roughly corresponding to the position of the pre-existing dark-etching rim. A boundary is visible between the unaltered, parental zoned γ -taenite (γ_p) and the $\gamma_t + \alpha$ transformation (t) products. In general, α -kamacite precipitates have developed perpendicular to the reaction boundary.

Microprobe analyses of the transformation products give γ_t -taenite with 49–51 wt% Ni and α -kamacite with ~5 wt% Ni, and there is a discontinuous change in the γ Ni concentration across the γ_t/γ_p reaction front (Fig. 2). The plessitic transformation is a discontinuous precipitation reaction of the type²



Compositions of the transformation products indicate equilibrium precipitation at a temperature of 350 °C (ref. 12), and

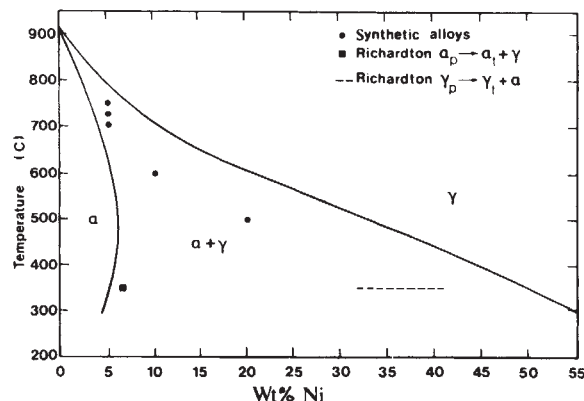


Fig. 3 The Fe-Ni equilibrium phase diagram (ref. 12) showing alloy compositions in which discontinuous precipitation reactions have been synthesized in the laboratory (ref. 2), and Richardton parental (α_p) kamacite (ref. 3) and zoned γ -taenite (this work).

proportions of γ -taenite to α -kamacite appear sufficient to maintain a Ni mass balance between the parental γ -taenite and the transformed regions at 350 °C (Fig. 3).

The structure of discontinuous plessitic transformations in Richardton indicates nucleation at, and inward growth from, high Ni, metal-silicate grain boundary regions of zoned γ -taenite grains. Axon and Grokhovsky³ have suggested localized mild grain boundary cold working, followed by reheating to 350–400 °C as a mechanism for discontinuous precipitation in Richardton α -kamacite. In addition, they note that the lack of Neumann bands indicates that the deformation rate was low. It appears that reheating in Richardton was not generated internally by shock and may have been external. Stress nucleation of the transformation during the later stages of cooling (400–350 °C) from high temperatures is another possibility, and one which obviates the need for an external heat source. However, irrespective of the thermal history, it is clear that initiation of the discontinuous precipitation reaction in α -kamacite was stimulated by strain, because locally there are unrecrystallized strain-lines in metal abutting silicates³. No evidence of strain was observed in Richardton γ -taenite; however, this is inconclusive because the f.c.c. γ structure shows few microscopically visible effects at low strain intensities^{13,14} and it appears likely that the plessitic transformation was initiated by strain effects at γ -taenite-silicate interfaces.

Discontinuous precipitation reaction in Richardton's zoned γ -taenite is less extensive and more localized than that observed in its kamacite³. Plesitic transformations do not occur in all zoned γ -taenite grains and are absent along γ -taenite grain boundaries which abut α -kamacite and troilite. Presumably, some γ -taenite grains were protected from the effects of strain by the volumes of crystalline silicates in which they are embedded and locally, γ -grain boundaries were similarly protected by abutting α -kamacite and troilite. Variable effects such as this are common in chondritic materials which are known to respond heterogeneously to mechanical deformation¹⁴.

The partial, grain boundary plessitic transformations in Richardton γ -taenite contrast with the γ -core plessitic decompositions normally encountered in meteorites¹³. In most reheated chondrites the process of plessite formation has gone to completion^{15,16}. Metal in Richardton is interesting in that discontinuous precipitation reactions have been interrupted and have not obscured features acquired during slow cooling from high temperatures. Low temperature $\gamma \rightarrow [\gamma + \alpha]$ discontinuous reactions similar to those in Richardton have also been observed (by V.J.G.) in the Plainview (1917) (H5) chondrite, where heating was undoubtedly generated by shock¹⁷. Strain-nucleation and discontinuous precipitation of the type observed in Richardton γ -taenite may be an important mechanism for the production of plessite in meteorites, particularly those for which heating after strain, or shock reheating, is inferred.

Discontinuous precipitation reactions of the types $\alpha \rightarrow [\alpha + \gamma]$ and $\gamma \rightarrow [\gamma + \alpha]$ have been synthesized in Fe,Ni alloys at temperatures $\geq 500^\circ\text{C}$ in the laboratory² (see Fig. 3). In Richardton, we now have evidence of two naturally occurring discontinuous precipitation reactions at lower temperatures (350°C) and, in the case of Richardton zoned γ -taenite, at higher Ni alloy concentrations.

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Rare-earth distributions with a positive Ce anomaly in the Western North Atlantic Ocean

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From the vertical distributions of the rare-earth elements (REE) in the Sargasso Sea we now report the first profiles of Pr, Tb, Ho, Tm and Lu in seawater, together with profiles for La, Ce, Sm, Eu and Yb. The first observations of positive Ce anomalies in seawater are ascribed to reducing inshore sediments as a source for Ce (ref. 1). All vertical profiles are consistent with adsorption of trivalent rare earths by settling particles, possibly siliceous or calcareous, followed by their release at or near the sea floor on dissolution of the carriers. The very different Ce profile demonstrates the additional effects of oxidation-reduction reactions.

The work reported here, earlier studies of Nd/Sm isotope systematics^{2,3}, and the recently reported profiles⁴ of nine REE, demonstrate the renewed interest in the REE in the oceans. All REE exist essentially in the trivalent oxidation state, and their chemical properties vary gradually along the series⁵. However, unlike the other REE, Ce can be oxidized to a highly insoluble⁶ tetravalent state, and reduction of europium to a divalent state may also occur. Although many other trace elements in seawater such as Mn (refs 7, 8), Fe (refs 8, 9), Cr (ref. 10), As (ref. 11), Sb (ref. 11), Se (ref. 12) and I (refs 13, 14) are affected by their multiple oxidation states, Ce and Eu are unique, because anomalies can be quantitatively defined by comparison with their neighbours within the REE series. Thus one can potentially single out oxidation-reduction reactions of Ce and Eu from all other processes affecting their distributions.

Seawater samples (4 l) were collected in August 1980. The water was pumped through a CHELEX-100 chromatography column for extraction of REE and separation from the major ions¹⁵. After subsequent purification by cation¹⁶ and anion¹⁷ exchange, the samples are analysed with neutron activation followed by γ spectrometry on a Ge(Li) detector. An overall chemical yield of 100.0% was demonstrated with radiotracer experiments. Precision (1σ) estimates for La, Ce, Sm, Eu, Tb,

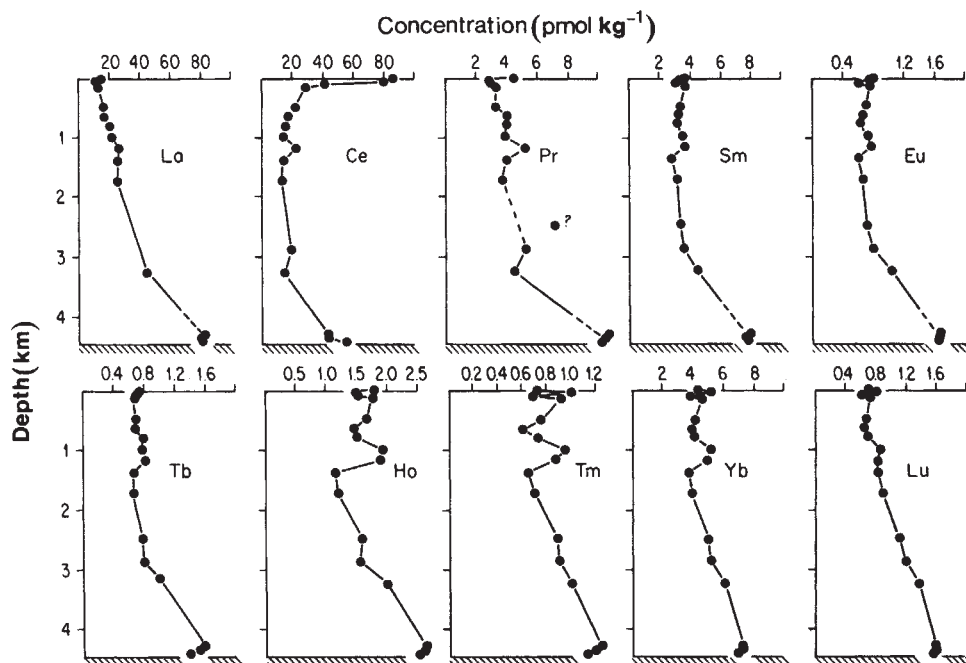


Fig. 1 Profiles versus depth of rare-earth elements in the north-west Atlantic Ocean. Some of the REE show a maximum at 1,179 m depth where the hydrography is particularly complicated^{19,21,23,45}. Mediterranean overflow water has occasionally been observed at similar depths in this region⁴⁵, but it is not clearly shown in our hydrographic data.

Yb and Lu range from 2 to 5%. Full details of methods and reproducibility will be reported elsewhere.

The first detailed profiles for Pr, Tb, Ho, Tm and Lu, as well as profiles for La, Ce, Sm, Eu and Yb are shown in Fig. 1. Concentrations of Lu, Tm and Tb, as well as Eu (ref. 4), are typically $<1 \text{ pmol kg}^{-1}$ and are the lowest reported thus far¹⁸ for any non-radioactive element in seawater. The vertical profiles, except that of Ce, exhibit a consistent, fairly linear, increase with depth, as also observed in the eastern North Atlantic⁴. At both locations the lightest REE exhibit the strongest gradient with depth. The increasing trend with depth bears a faint resemblance⁴ to the distribution of nutrients, especially silicate. However, the following dissimilarities suggest that most of the processes governing the REE transport cycle are different from those controlling the nutrients. In the upper waters the REE and nutrient profiles diverge appreciably, the nutrients decreasing to very low values (not shown), whereas the REE continue their linear trend all the way up to the sea surface (Fig. 1). Thus any incorporation of the REE into biogenic phases does not seem to exert a dominant control on their vertical distributions. For depths below 1,000 m, a plot of the heaviest rare earth, Lu, versus silicate (not illustrated) showed a linear relationship with a strong positive correlation ($r = 0.997$, $P < 0.001$). Because silicate is an approximately conservative tracer in the deep north-west Atlantic¹⁹⁻²³, this result suggests that removal of Lu is slow compared with local renewal rates of the deep water masses. However, plots of the other REE versus silicate showed increasingly negative curvature with decreasing atomic number, indicative of efficient scavenging for the lighter REE relative to Lu. This observation and the heavy REE enrichment discussed below are consistent with adsorptive scavenging²⁴⁻²⁶ over the entire water column, combined with a release at or near the sea floor. The noted⁴ similarity with some profiles of Cu and Al, as well as Be, is also compatible with these abiotic processes^{18,27-29}.

Our Ce profile differs dramatically from that reported by Elderfield and Greaves⁴ and the profiles of the other REE. It shows high values in the mixed layer, a rapid decline to a minimum at mid depth, and then again an increase towards the sea floor (Fig. 1). Further discussion of our results for Ce and the other REE is best done with reference to their relative abundance patterns (Fig. 2) which exhibit three major features.

(1) An enrichment of the heavy relative to the light REE, in accordance with earlier observations^{4,30,31}. The Lu/La ratio in the upper water column is about four times higher than in

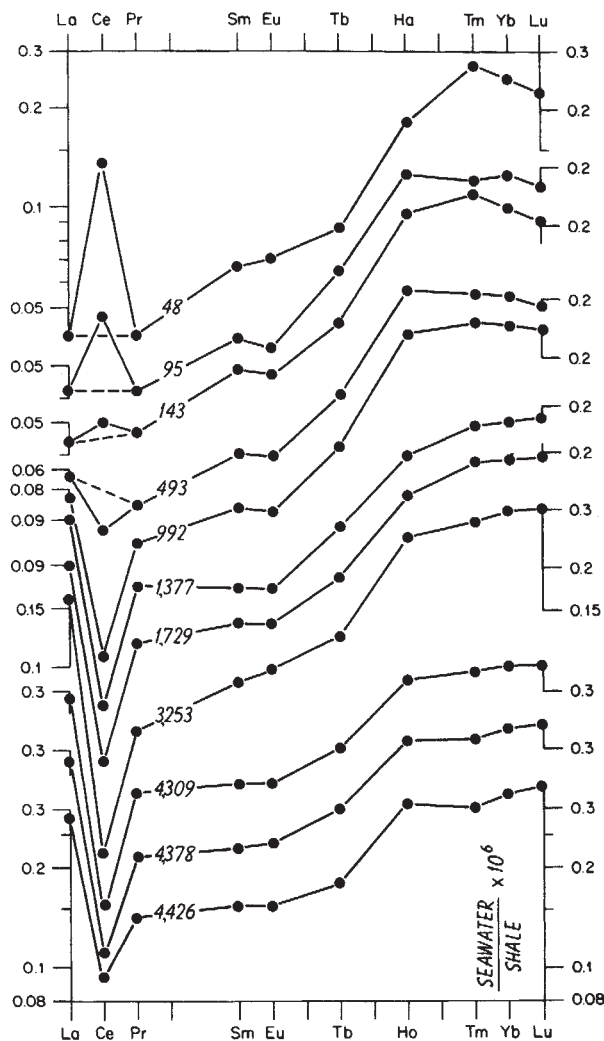


Fig. 2 Distribution patterns of REE normalized versus shales³³ at selected depths. Offset vertical log-scales. Values for shales are thought to represent average abundances in the continental crust⁵. The limited data base (ref. 4, Table 1), and the virtual absence of data for seawater dissolvable REE inputs (aeolian^{34,36}, riverine³⁷, hydrothermal?), do not yet allow definition of a more suitable REE normalization standard.

Table 1 Total 'dissolvable' concentrations (10^{-12} mol per kg seawater) of rare-earth elements at a 4,460 m deep Sargasso Sea station (33°58' N, 58°05' W)

Depth (m)	La	Ce	Pr	Sm	Eu	Tb	Ho	Tm	Yb	Lu
10	15.0	86	4.5	3.7	0.78	0.75	1.8	0.74	4.3	0.68
49	12.0	80	2.95	3.35	0.75	0.73	1.5	1.0	5.1	0.78
98	12.3	42	3.0	3.0	0.60	0.69	1.55	0.675	3.8	0.61
147	12.9	30	3.4	3.65	0.75	0.68	1.8	0.925	4.6	0.72
491	16.7	23	3.4	3.4	0.70	0.69	1.7	0.76	4.1	0.66
638	17.8	18	4.05	3.2	0.65	0.68	1.5	0.615	3.9	0.64
783	21.3	16	4.0	3.2	0.64	0.79	1.5	0.73	4.05	0.68
981	22.2	15	4.0	3.5	0.73	0.77	1.9	0.95	5.1	0.85
1,179	27.2	23	5.3	3.6	0.76	0.82	1.9	0.88	4.9	0.82
1,379	26.2	15	4.1	2.8	0.60	0.67	1.8	0.655	3.7	0.83
1,719	26.2	14	3.8	3.05	0.65	0.65	1.2	0.70	3.9	0.88
2,486	—	—	(7.2)	3.3	0.72	0.78	1.6	0.89	5.0	1.10
2,874	—	20	5.3	3.5	0.80	0.80	1.6	0.90	5.2	1.17
3,264	46.6	16	4.6	4.5	1.04	0.97	2.0	1.03	6.1	1.36
4,328	83.8	44	10.7	7.9	1.67	1.57	2.65	1.27	7.3	1.59
4,378	80.8	44	10.4	7.6	1.66	1.53	2.6	1.21	7.35	1.59
4,427	82.2	55	10.3	7.75	1.65	1.40	2.5	1.14	6.95	1.54

Samples were left unfiltered to avoid contamination. The suspended particulate REE fraction has probably an order of magnitude lower concentration³⁶, was probably retained on the Chelex columns and was thus excluded from our analyses. Extractions and separations were done in a Class 100 clean air room using hot acid leached PE, Teflon and Pyrex labware. Our values for La, Ce, Sm, Eu and Yb are in agreement with the concentration ranges recently reported by others using different methods^{2,4,44}.

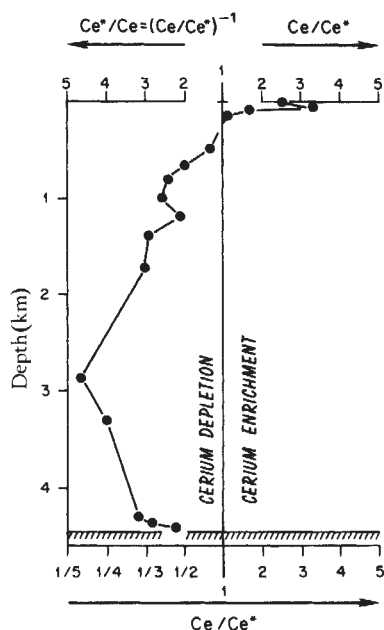


Fig. 3 Profile versus depth of the Ce anomaly defined as Ce/Ce^* (see text). Enrichment (positive anomaly) plotted as Ce/Ce^* , depletion (negative anomaly) as its inverse Ce^*/Ce .

shales. Heavier REE are believed to be stabilized in seawater due to formation of stronger inorganic complexes³², so the lighter REE are expected to be scavenged more efficiently^{25,26} by adsorption on settling particles. This is the same effect as inferred from our comparisons with silicate discussed above and was first suggested by Goldberg *et al.*³⁰. One would expect sinking particulate matter to exhibit light REE enrichments, but this has yet to be verified. With Ce disregarded, the REE patterns (Fig. 2) in the upper 1,000 m are very similar. However, from about 1,000 m downwards a minimum tends to develop at about Sm and Eu. This could be due to injection of a light REE enriched fraction released from dissolving biogenic particles at or near the sea floor. Most probably the relative rates of adsorption and desorption determine the shape and location of the minimum in the REE pattern for a given parcel of water.

(2) Absence of a significant Eu anomaly at all depths. Concentrations of Eu and Sm are almost perfectly correlated ($r = 0.998$, $P < 0.001$), with a ratio $Sm/Eu = 4.6$, close to the value of 4.7 for shales³³. The small Eu anomaly reported recently⁴ appears to be related to a similar anomaly in a terrestrial aerosol source³⁴. Thus far there is no clear evidence for reduction of Eu in seawater, although one might expect Eu anomalies to develop in strongly reducing environments such as anoxic basins or hydrothermal vents³⁵.

(3) The first observation of distinct positive Ce anomalies in seawater. Our neutron activation procedure yields data for both adjacent elements in the series, La and Pr, thus allowing us to define this anomaly quite precisely:

$$Ce \text{ anomaly} = Ce/Ce^* = 2(Ce/Ce_{\text{shale}})/(La/La_{\text{sh}} + Pr/Pr_{\text{sh}})$$

where Ce^* is the hypothetical concentration that a strictly trivalent Ce would have, as interpolated from La and Pr. In the mixed layer Ce is two to three times enriched (positive anomaly) relative to La and Pr. The anomaly drops off rapidly with depth until the transition from positive to negative values at ~250 m (Fig. 3). The ratio Ce/Ce^* then gradually diminishes to produce the well known^{4,30,31} three- to fourfold depletion at depth. The negative Ce anomaly indicates enhanced removal of dissolved trivalent Ce as a result of oxidation to a highly insoluble⁶ tetravalent state. In principle one can quantify the net effect of this oxidation by using a modified version of the vertical scavenging model²⁴. Scavenging rate constants $\psi_{\text{REE}}/\psi_{\text{Lu}}$ relative to the most conservative element Lu can be assessed. Thus various assumptions (for example, an absolute value of the upwelling velocity w) necessary for derivation of an absolute scavenging rate constant ψ_{REE} (yr^{-1}) are not required. Preliminary calculations for the deep water (>1,000 m) suggest that La and Pr are removed about twice as fast as Lu, but that Ce is removed about four times as fast as Lu. From the following relationship

$$\frac{\psi_{\text{Ce total}}}{\psi_{\text{Lu}}} - \frac{\psi_{\text{Ce oxid}}}{\psi_{\text{Lu}}} = \frac{\psi_{\text{Ce diss}}}{\psi_{\text{Lu}}} = \frac{1}{2} \left(\frac{\psi_{\text{La}}}{\psi_{\text{Lu}}} + \frac{\psi_{\text{Pr}}}{\psi_{\text{Lu}}} \right)$$

it is thus suggested that the change in oxidation state approximately doubles the removal efficiency for Ce at our station.

The positive Ce anomaly in surface waters is a new observation. The vertical profiles of Ce (Fig. 1) and especially the Ce anomaly (Fig. 3) are strikingly similar to open ocean profiles of Mn (refs 7, 8), suggesting that similar mechanisms govern both elements. The surface maxima found for Mn have been

attributed to either aeolian⁷ (or riverine⁸) inputs or an injection from reducing inshore sediments⁸. In the eastern North Atlantic there is good evidence⁴ for an aeolian input of REE, as characterized by a shale type pattern. At our station, however, an aeolian input would have to be enriched with both Ce and heavy REE in order to match the observed pattern in the surface waters (Fig. 2). This is conceivable, but there is no evidence supporting this hypothesis. The current, very limited, data for aerosols^{34,36} exhibit shale type patterns instead. In analogy the only available data for rivers also exhibit shale type patterns³⁷, although fractionations may very well occur during estuarine mixing. For instance, the fluvial component of inshore sediments might exhibit a positive Ce anomaly.

Cerium anomalies, both positive and negative, have been observed almost exclusively within the marine environment. Therefore Ce fractionations are more likely to be the result of strictly marine processes. The following explanation of the observed Ce enrichments in surface waters depends entirely on such cycling of Ce within the ocean basins. All trivalent REE, including Ce³⁺, are removed from seawater by adsorption. Additional removal of dissolved Ce(III) by oxidation to solid Ce(IV) is suggested by the calculations described above and the well documented Ce depletion of deep waters in other areas^{4,30,31}. One would expect to find a positive Ce anomaly for the authigenic fraction of particles settling towards the sea floor, but this has yet to be verified. Post-depositional reduction to Ce(III) may occur in organic rich inshore sediments, at which point Ce behaves again as the other, strictly trivalent, REE. Nevertheless, a regenerative flux of all REE (including Ce³⁺) from these sediments would still carry the memory of a positive Ce anomaly. Relatively slow oxidation rates^{38,39}, in analogy with manganese⁴⁰, would allow some diffusion of dissolved Ce(III) across oxic surface sediment layers and subsequent lateral transport into the centre of the basin, leading to the observed enrichments in the surface waters. Then margin sediments would act as a source for both the positive anomaly and Ce itself. In surface water transects one expects both these properties, like Mn (ref. 8), to decrease in an offshore direction as a result of oxidative scavenging removal. Similar trends have been demonstrated⁴¹ for ²²⁸Ra, the latter being removed by radioactive decay^{42,43}.

The sharp gradient of the Ce anomaly near the sea floor (Fig. 3) may be ascribed to essentially the same mechanisms. Studies of REE distributions in surface water transects, pore waters and anoxic basins are necessary for further testing of the above mechanisms.

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Accumulation of nitrous oxide in the oxygen deficient layer of freshwater lakes

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Nitrous oxide (N₂O) is an intermediate in denitrification (dissimilative nitrate reduction)¹ and a by-product of nitrification². In the ocean, the N₂O producing mechanism is thought to be mainly nitrification^{3,4} and in an Antarctic lake Vincent *et al.*⁵ recently presented evidence that the depth of maximum nitrifying activity coincided with that of maximum N₂O concentration. In soils it has been confirmed that N₂O is produced through denitrification⁶. However, in natural waters knowledge of N₂O accumulation is still very limited.

In two freshwater lakes in central Japan, we have found large amounts of N₂O to be accumulated in layers near the bottoms where O₂ concentrations were about 0.1 ml l⁻¹. We suggest here that such N₂O accumulation is due to a denitrification process.

In a study of Lake Kizaki, waters were sampled by lowering a 3-l flask into the lake. N₂O was flushed out from the water, and adsorbed on a Molecular Sieve 5A at a field laboratory near the lake using a modified form of Hahn's procedure⁷. N₂O was then determined by TCD-type gas chromatograph in the university's laboratory. In the case of Lake Fukami-ike, each sample of about 100 ml water was obtained in the vial, and preserved at 5 °C after injection of 0.3 ml of saturated HgCl₂ solution and sealing with a butyl stopper and an aluminium cap until N₂O determination. In the laboratory, an aliquot of sample (1–50 ml) was injected into the flushing column with syringe. N₂O was adsorbed on Molecular Sieve 13X first, desorbed by heating, and determined by ECD-type gas chromatograph following Cohen's method⁸. O₂ was determined by Winkler titration, nitrite by the method of Bendschneider and Robinson⁹, and nitrate following Wood *et al.*¹⁰.

Lake Kizaki is a mesotrophic lake having a maximum depth of 28.5 m and an area of 1.4 km². Its oxygen is depleted near the bottom from late summer to late autumn. On 11 October 1981, a maximum concentration of N₂O, 3.79 µg atom l⁻¹ (133-fold air-equilibrium concentration), was found at 25.0 m depth where O₂ concentration was 0.13 ml l⁻¹. At 27.5 m depth, however, the N₂O concentration decreased to 0.004 µg atom l⁻¹ (much lower than the air-equilibrium concentration) and O₂ was completely absent (Fig. 1).

An accumulation of N₂O in deeper layer was also found in Lake Fukami-ike, a eutrophic lake with a maximum depth of

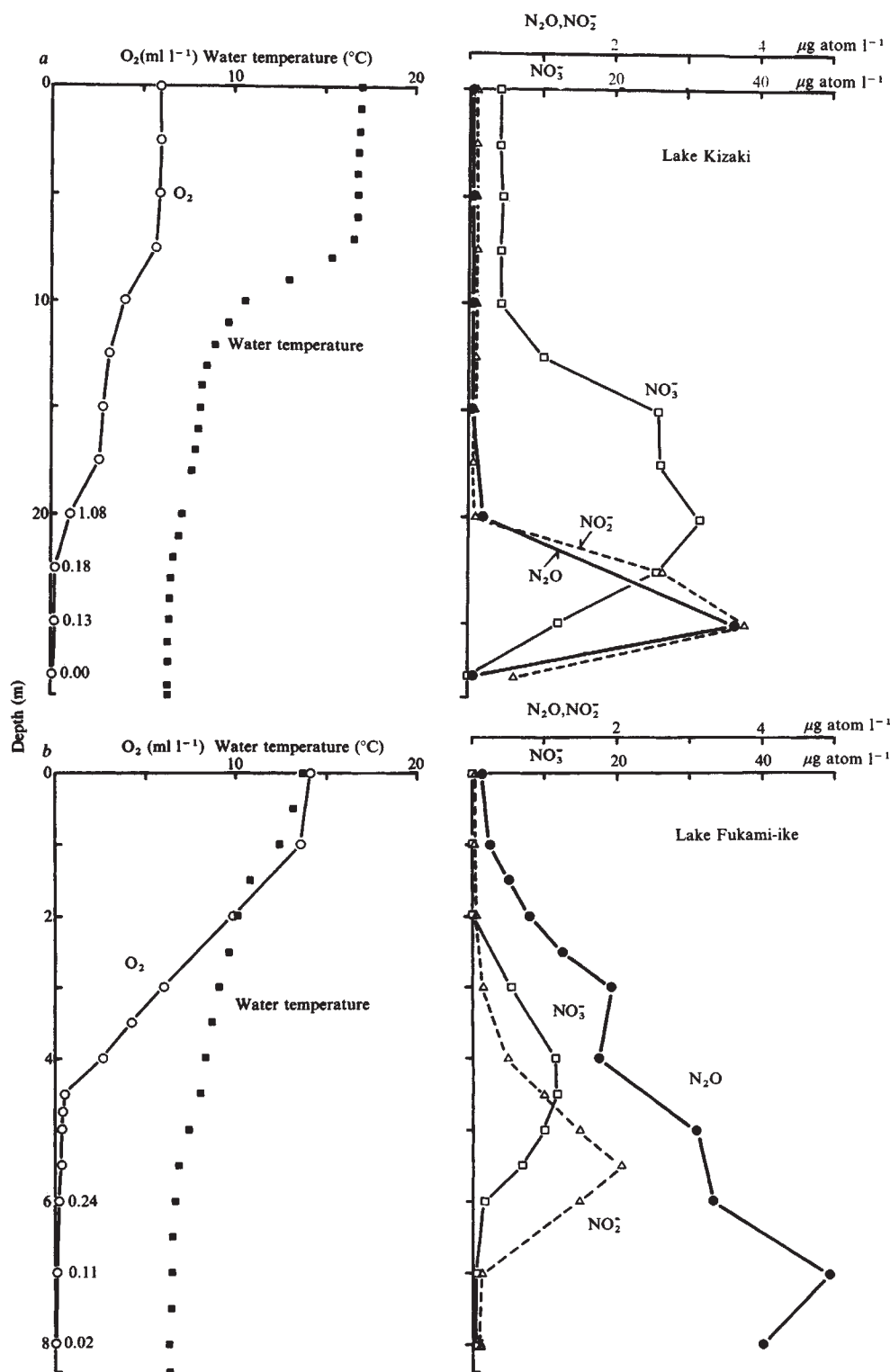


Fig. 1 Vertical distributions of water temperature (■) and O₂ (○), N₂O (●), NO₃⁻ (□) and NO₂⁻ (Δ) concentrations in Lake Kizaki on 10 October 1981 (a), and in Lake Fukami-ike on 2 April 1982 (b). The air-equilibrium concentrations of N₂O ranged 0.020–0.029 μg atom l⁻¹ in these two waters. Values of oxygen concentration in deeper layer are shown.

8.5 m and an area of $2.2 \times 10^4 \text{ m}^2$. In the deeper layer, below ~4 m, the anoxic condition prevails from spring to autumn. On 3 April 1982, a month or so after the spring overturn of this lake, N₂O increased from the surface towards the bottom. The highest amount of N₂O, $4.88 \mu\text{g atom l}^{-1}$ (174-fold air-equilibrium concentration), was found at 7.0 m depth where O₂ concentration was 0.11 ml l^{-1} . At a depth of 8.0 m just above the bottom, N₂O decreased slightly to $4.04 \mu\text{g atom l}^{-1}$, and the O₂ concentration was 0.02 ml l^{-1} (Fig. 1).

Such low concentrations of O₂ at the N₂O-accumulating layers are thought to favour denitrification. In addition, the

sequential distributions of peaks of NO₃⁻, NO₂⁻, and N₂O concentrations with depth were observed in both lakes. A similar profile of NO₃⁻, NO₂⁻ and N₂O associated with denitrification was observed in coastal marine sediments by Sørensen¹¹. Furthermore, at 25.0 m depth in Lake Kizaki, NO₃⁻ concentration decreased from $35.5 \mu\text{g atom l}^{-1}$ to $16.5 \mu\text{g atom l}^{-1}$, from 10 August to 11 October. In the deeper layer of Lake Fukami-ike, also, a decrease of NO₃⁻ concentration must have occurred since the spring overturn. Recently, Knowles *et al.*¹² observed a large amount of N₂O in the spring in a O₂-depleted hypolimnion of a eutrophic lake and suggested that denitrification contributed

to its accumulation based on the NO_3^- decrease in that body of water. These facts indicate that the N_2O accumulations in the two lakes studied can be attributed to denitrification.

On the other hand, several reports on N_2O distribution in some natural water bodies have demonstrated that N_2O was nearly or completely absent where the O_2 concentration was nil¹³ or extremely low, near the detection limit of O_2 by Winkler method¹⁴, and it was considered that N_2O depletion was caused by the denitrification.

Thus, we conclude that the optimal O_2 concentration in which N_2O is accumulated through denitrification appears to be about 0.1 ml l^{-1} in these lakes. Below this O_2 level, the N_2O reduction rather than its production seems to be accelerated (at 27.5 m in Lake Kizaki), as discussed by Betlach and Tiedje¹⁵.

The accumulation of N_2O with only a trace amount of O_2 in the deeper layer could be found in most lakes where the O_2 depletion take place during the period of stratification.

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Vertical open burrows in deep-sea sediments 2 m in length

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Previous studies of the mixed layer, in deep-sea sediments suggest a maximum burrowing depth of about 10–30 cm (ref. 1). However, we now report that two recently obtained cores from the north-east Atlantic (S126/2 and S126/4) display open burrows to a depth of 214 cm within a distal pelagic turbidite. An organic lining in one of the burrows indicates a modern origin. Many of the burrows are less than 0.5 mm across and are only visible on fractured surfaces. Their effects on sediment mixing are probably minimal, but they provide a network of open vertical channels which considerably increases the permeability. This is of crucial importance to studies involving pore water movement, such as those associated with the radioactive waste disposal project. The effect of the burrows changes the calculated permeability from the equivalent of a clay to that of a coarse sand. The delicate structure of the burrows may result in their closure during handling, thus requiring their effect on pore water fluxes to be studied *in situ*.

The open burrows were observed on fracture surfaces of two Kastenlot² gravity cores taken on the Madeira abyssal plain (Tables 1, 2). The corer, a 4-m long square box, is known to recover sediments with a minimum of disturbance. Each core consists of a thick calcareous clay unit (distal pelagic turbidite), overlain by thin layers of pelagic sediment and a second thin turbidite (Fig. 1). The lower part of the thick turbidite is greenish in both cores indicating reducing conditions, but its upper half is pale buff coloured indicating oxidizing conditions.

Two types of open burrow were observed. The first—about 5 mm in diameter (Fig. 2)—is often surrounded by a discoloured

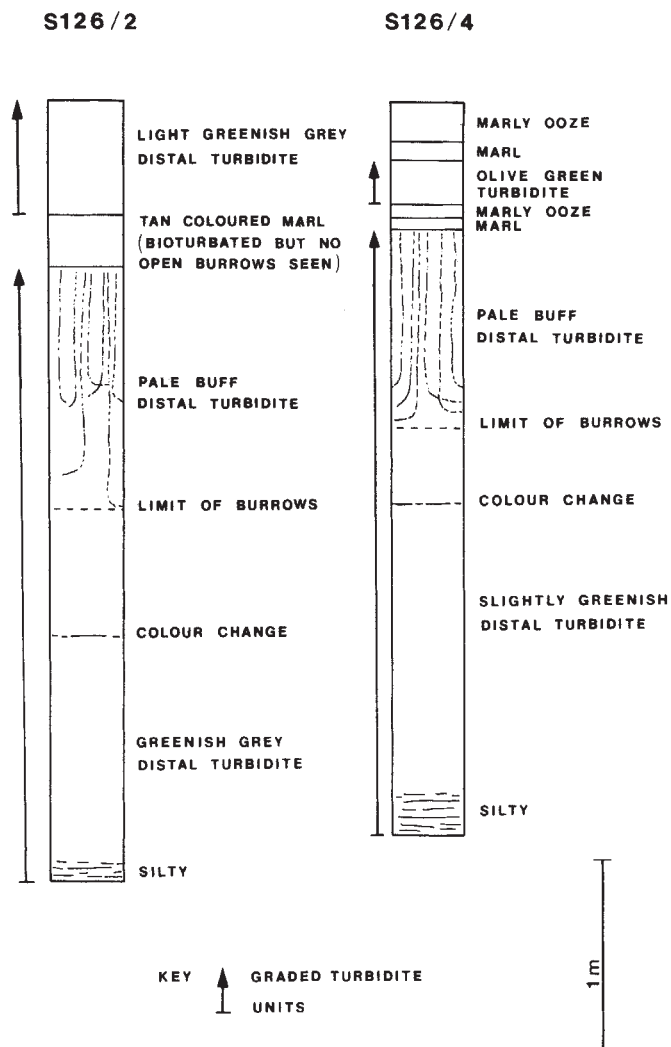


Fig. 1 Lithology and distribution of *Trichichnus* open burrows in cores S126/2 and S126/4. Burrows indicated by irregular dashed lines.

pinkish halo. These are quite rare, but run in both vertical and horizontal directions. They were found to a maximum depth of 115 cm. Similar burrows have been described in cores from the equatorial Pacific³.

The second type of open burrow which has been found to a maximum depth of 214 cm in core S126/2 (170 cm in S126/4) is much narrower, up to 1.2 mm but commonly less than 0.5 mm in diameter (Fig. 2). These probably belong to the genus *Trichichnus*⁴ being long, thin and numerous, with up to 50 per 100 cm². The burrows must post-date the turbidite which is deposited very quickly and hence the minimum length of individual burrows is about 70 cm in core S126/2 and 100 cm in core S126/4 (Fig. 1). These burrows are too delicate to be revealed by conventional core splitting techniques. They were not visible on surfaces cut later with an electro-osmotic knife, and even the burrows revealed on the fracture surface became unrecognizable within 1–2 h of exposure. *Trichichnus* burrows were only found in the thick turbidite, possibly because the overlying sediment is too plastic to preserve them. In core S126/2 they are all vertical and linear to a depth of 150 cm, below which they become horizontal (Tables 1, 2). Over three-quarters of the horizontal burrows are sediment filled (Fig. 2b); this infilling often continues into the lowest 20–30 cm of the vertical sections. However, most remain open in their upper sections. The filled burrows are wider than their open equivalents (Fig. 2) either due to material falling from the walls during the filling process or because the open burrows have

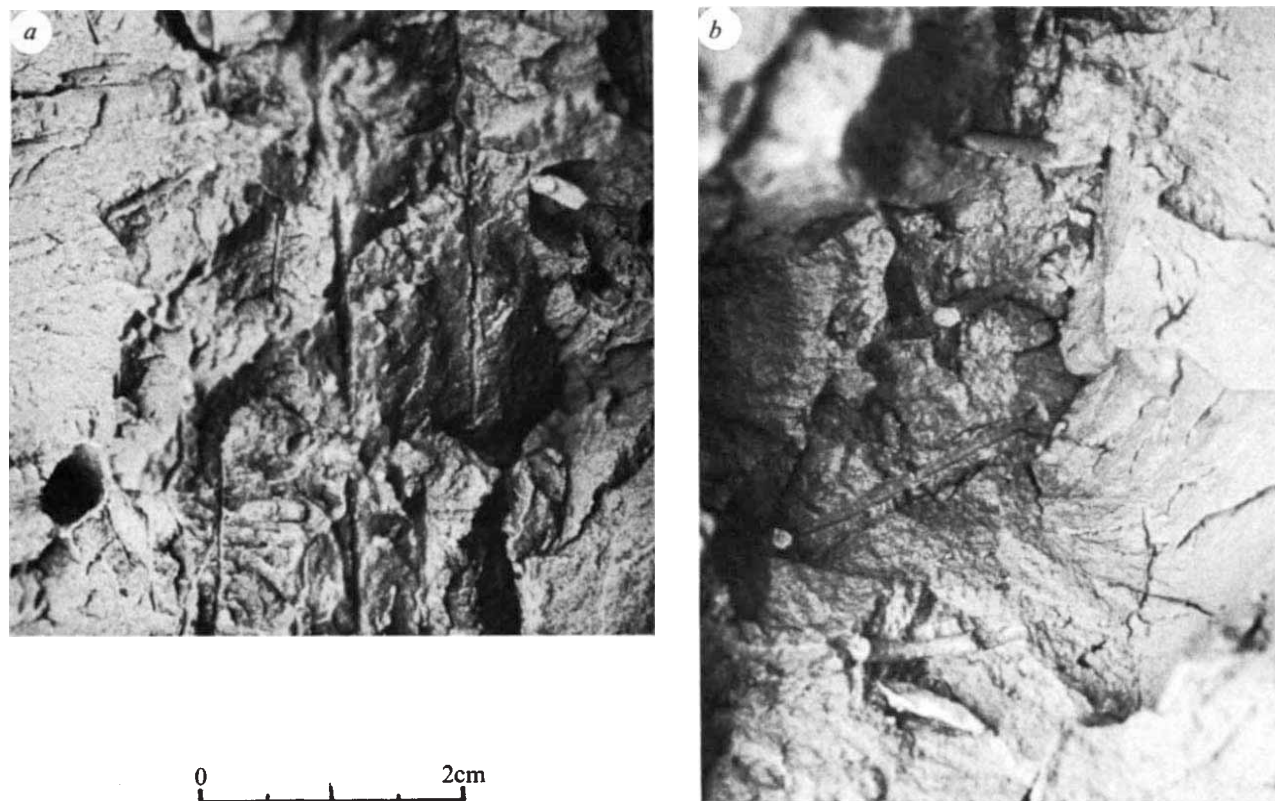


Fig. 2 a, Large open burrow and numerous vertical, open *Trichichnus* burrows on fractured surface of core S126/2. b, Horizontal filled *Trichichnus* burrows in core S126/2.

Table 1 Location of cores

Core no.	Latitude	Longitude	Water depth (corr m)	Core length (m)
S126/2	31°33.2'N	24°50.5'W	5,444	4.15
S126/4	31°31.7'N	24°25.3'W	5,446	3.83
D10311	25°39.2'N	30°57.6'W	6,129	2.17

Table 2 Burrow density and attitude in S126/2

Depth from sediment surface (m)	No. of vertical burrows per 100 cm ²	No. of horizontal burrows per 100 cm ²
150	50	0
166	14	10
170	5	2

partially closed during coring. The open burrows are difficult to identify on X-radiographs probably due to burrow closure. However, a few have been observed on X-radiographs through 2-cm thick slices. Similar features have also been observed in core D10311 (Tables 1, 2) which consists of red clay above a distal pelagic turbidite. The open burrows here occur at the base of the red clay.

One of the *Trichichnus* burrows, between 143 and 149 cm in core S126/2, contained a thin, flexible, organic tube representing the mucous lining of a worm burrow. Either pogonophores or polychaetes⁵ could be responsible for the formation of such burrows. The organic lining indicates that the particular burrow was recently inhabited rather than having an ancient origin. The organisms are, therefore, capable of burrowing through the softer overlying sediment, even though no burrows were observed in this facies. The vertical length of individual burrows could therefore be as long as 214 cm. The presence of reduced, green-coloured sediment beneath the burrows could

be significant in that bacteria, on which both groups feed, may preferentially exploit sediment near to a redox boundary. Conversely the sediment may be oxidized by water flow through the open burrows. No organic carbon-rich layers were found in the core.

How widespread such burrows are in the deep sea is not known. If the organisms are exploiting sediment because of the nearby redox boundary, then they may be restricted to sediments with reduced layers. Similar burrows have been recorded in red clays from the Cape Basin⁶, and in sediments from the north-west African continental margin⁷.

Open burrows in deep-sea sediments have a profound effect on the overall permeability, and consequently, on the possible flow rates through them in response to any excess pore pressures. It can be shown that, for a mass of sediment containing smooth open parallel tubes (assuming Darcian and Poiseuille flow in the sediment and tubes respectively), the absolute permeability in the direction of the tubes, K (cm²) is given by:

$$K = K_s(1 - n\pi r^2) + n\pi r^4/8$$

where K_s is the absolute permeability of the unburrowed sediment (cm²), n is the number of tubes, per square centimetre, r is the radius of the tubes (cm).

Figure 3 illustrates the influence of open burrows on the permeability of deep-sea clays using the above equation for a range of values of r and n . K_s becomes negligible in the above equation when the values fall below 10⁻¹⁰ cm². Most deep-sea sediments have permeabilities ranging between 10⁻¹⁰ and 10⁻¹² cm², so the increase by two orders of magnitude produced by the open burrows (where $n = 0.5$ and $r = 0.2$ mm) becomes most significant. In red clays, with typical permeabilities of 10⁻¹² cm², the effect would be to increase the permeability by four orders of magnitude.

Laboratory measurements on these cores give K_s values around 10⁻¹¹ cm² for the interval below the open burrows

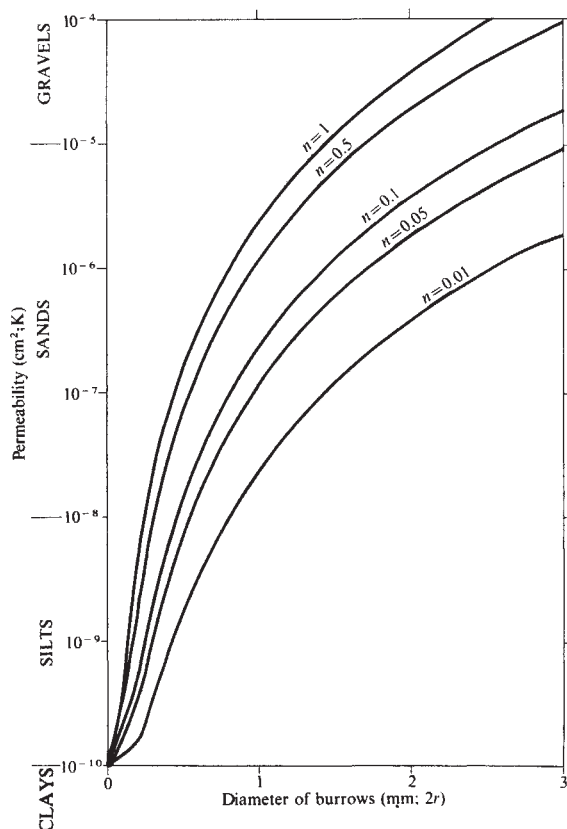


Fig. 3 Effect of open burrow size and density on the permeability of deep-sea clays. Calculated assuming clay permeability = 10^{-10} cm². Generalized permeability ranges shown for comparison.

(P.J.S., unpublished data). In the burrowed interval, however, values of 10^{-10} cm² were recorded. The reason for values much lower than those predicted in Fig. 3 may be twofold. Permeability measurements at low effective stresses are often inaccurate due to sample sealing problems and such delicate burrows may close, at least partially, during sampling.

The existence of long open burrows and their effects on sediment permeability are significant to studies such as the radioactive waste disposal programme, sediment stability studies and investigation into advection/diffusion⁸ of ions through sediments. It is interesting to speculate that some of these burrows may be kept open by vertical pore water advection within the sediment⁹. Although we have no direct evidence of this effect, the observation that only horizontal burrows are sediment filled supports this hypothesis. The difficulty of making laboratory measurements necessitates that *in situ* measurements are made in the future.

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Salting of food—a function of hole size and location of shakers

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The establishment of an association between hypertension and the level of sodium in the diet¹⁻² has focused interest in many countries on the amount of salt added to food, with estimates of intake in western countries being in the range 6-14 g per person per day^{3,4}. As a result, many health authorities have advocated a decrease in salt consumption by the population in general⁵, a common suggestion for achieving a meaningful reduction being to limit the amount of salt used at the table⁶. It is generally assumed that salting habits are influenced by taste preference as shaped by socio-cultural determinants. However, from our observations of the rate of discretionary salt usage of over 1,900 people (mainly adults) consuming main meals in public institutions in Sydney, Australia, we suggest that salting is strongly influenced by the physical factor of mode of presentation of salt to the consumer, particularly the hole size of the salt shaker, and is not influenced by demographic variables.

Data on the effect of hole size were collected from 586 adults patronizing a public cafeteria in a large department store by placing on the tables pre-weighed single-holed salt shakers, identical except for the size of the hole, and re-weighing the shakers after use. Figure 1 shows that for the individuals who added salt, the amount of salt used (*y* in g) was linearly related to the area of the shaker hole (*x* in mm²) ($y = 0.07x + 0.17$, $P < 0.001$). Data were also obtained for salt usage in a Boeing 747 cabin used regularly by an airline to train cabin stewards, with the 'passengers' (644 people) comprising community groups who were given a regular airline meal as part of a tour of the airline complex. The normal airline salt shakers (hole size 4.5 mm²) which initially contained 1.1 g salt were presented with the food and recovered after use for re-weighing. The amounts used by customers (385 individuals) in four medium-priced restaurants were also obtained by placing pre-weighed shakers of two hole sizes (4.9 mm² and 8.0 mm²) on the tables and re-weighing after use. The average amounts of salt used in the airline cabin and for the two hole sizes in the restaurants are shown in Fig. 1 and are consistent with the previously established linear relation ($P < 0.05$).

The effects of the method of salt presentation on salt usage were further examined in a study of 377 customers in five industrial canteens of a large public utility which serviced different occupational levels in the establishment. The salt was presented in three different modes: one canteen placed salt shakers on the table in the normal manner and average usage of these shakers (hole area 8.0 mm²) was 0.73 g per person. This value is consistent with the linear relation shown in Fig. 1 ($P < 0.05$). Three canteens which catered for different occupational profiles (blue-collar, skilled and white-collar workers) placed the salt shakers at the end of the food line. No differences were observed between venues and hence between occupational levels, but for the three canteens the average amount used was lower than in the first canteen: 0.53 g per user ($P < 0.05$). The fifth canteen placed paper sachets containing 1.1 g salt at the end of the food line and the average amount used was increased to 0.93 g per user ($P < 0.05$). This increase was reflected in the modal value, which was 0.3-0.4 g for the canteens using salt shakers but with the sachets increased to 1.0-1.1 g, that is, the total content of the sachet.

Thus, while an individual's decision to use salt may be based on some expectation of the saltiness of a particular food, the

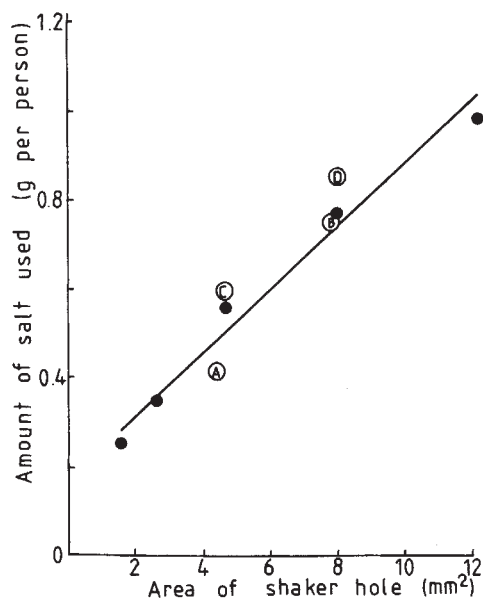


Fig. 1 Relation between amount of salt used and hole area of single-holed shakers placed on tables, in ●, cafeteria; A, airline cabin; B, canteen; C and D, restaurants.

amount actually added varies quite markedly with the mode of physical presentation of the salt. It seems, therefore, that in addition to lowering the levels of salt in foods⁷, salt intake could be reduced by presenting the salt in single-holed shakers in which the hole size is limited to 3 mm². A hole size of ~3 mm² seems to be the optimum as with smaller hole sizes some consumers attempted to make the hole larger with sharp implements such as forks. From the data in Fig. 1, a shaker of 3 mm² would give an average salt usage of ~0.37 g per person per meal, or ~1.1 g per day if it is assumed that three meals per day are salted equally. A survey of nine tableware suppliers in Sydney produced 81 different salt shakers of which 37 were single-holed with an average hole size of 4.9 mm² but with a size range of 2.7–8.3 mm² and 44 were multi-holed with an average total hole area of 18.7 mm² and a range of 4.0–44.7 mm². The overall average hole area of all the shakers was 13.0 mm² with an expected salt usage from Fig. 1 of ~1.1 g per meal or 3.3 g per day. However, as we did not examine multi-holed shakers, caution must be exercised in assuming that patterns of usage of multi-holed shakers parallel those of single-holed shakers. Placing the salt shaker at some distance from the table could be expected to further constrain salt usage and the provision of sachets is not recommended. A reduction in salt usage by manipulation of hole size and location of shakers would only apply to the 60% of persons who were found to add salt to their food. The range of salt added by individuals was large (0.1 g–2.3 g), hence many would be consuming well in excess of the average and for these persons manipulation of shaker usage could be expected to effect substantial decreases in consumption. The addition of salt would appear to be an habitual act as <25% of users tasted the food first. The level of usage was not affected by age or sex in any establishment.

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Radiation damage in L-valine at liquid helium temperature

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One of the most fundamental limitations to resolution in the electron microscopy of organic materials is the structural damage caused by the incident radiation. While the primary inelastic interactions between the electrons and the specimen molecules cannot be stopped, it has been hoped that the use of very low temperatures would 'freeze' molecular fragments in place to allow extended microscopy of minimally damaged structures. Knappek and Dubochet¹ have reported that the rate of damage is much less (30–300 times) when diffraction patterns of organic materials are observed in an electron microscope with a superconducting objective lens, in which the specimen is held at 4.2 K. Previous workers had not seen such an effect when using liquid helium cooled stages, and it was suggested¹ that this was mostly due to insufficiently low temperature. A microscope with a superconducting objective lens, which was constructed at Oak Ridge National Laboratory², has been installed recently at Duke University. We selected L-valine as a test material because it has been so extensively examined in the past. Knappek and Dubochet¹ reported that L-valine crystals at 4.2 K could tolerate an electron dose 67 times greater than at room temperature and Dietrich *et al.*³, using the same microscope, reported a tolerance 87 times greater; we report here that it is only 4 to 6 times greater. We have tested effects of temperature and of the specimen substrate, both suggested as important components in the limitation of radiation damage, and we find no further improvement. The results of Knappek and Dubochet and Dietrich *et al.* therefore remain unconfirmed, and the mechanism and extent of protection from radiation-induced damage at low temperatures must remain under study.

While earlier published measurements in the range of 10–20 K showed improvement of one to three times in resistance to radiation damage, there was good reason to believe that there might be a sharp increase in resistance at 4.2 K^{4,5} because some chemical bonds are maintained at this temperature despite irradiation, and atoms including hydrogen should be 'frozen' in position. The temperature in this situation would be quite critical, and it is desirable to work with an electron microscope with a superconducting objective lens where the specimen is surrounded by surfaces at liquid helium temperature. Our microscope has a very large and well-shielded cryostat⁶ in which the specimen is held at 4.2 K, and it was our first interest to measure independently the reduction in radiation damage at this temperature.

As the thermal and electrical conductivity of the specimen substrate was proposed to have an effect on the amount of radiation damage observed¹, we prepared L-valine crystals by evaporation of aqueous solution on several different substrates. Some crystals were placed on Formvar films on standard copper grids, and these grids were overcoated with carbon (made by Dr K. Taylor). Others were mounted on thin carbon films or thin titanium films over holey copper films.

For microscopy at liquid helium temperatures, the illuminating system was set to produce a uniform circle of ~5 µm diameter with a current of $\sim 3 \times 10^{-12}$ A, corresponding to an incident current density of $\sim 10^{-2}$ e Å⁻² s⁻¹. It is generally agreed that there is no problem with heating due to the beam or dose rate effects at this level of incident current density^{1,7}. The microscope was operated with an accelerating voltage of 65 kV. Magnifications were checked using a 2,160-line mm⁻¹

cross-grating replica. Images of the illumination spot were recorded at a magnification of 6,030 for each diffraction series and the dose rate was determined by film densitometry. Diffraction patterns were recorded in series of 45-s exposures followed by 15-s pauses for film changing. All recording was done on Kodak 4489 electron microscope film.

Room temperature measurements were done using a Siemens 102 electron microscope at 60 kV, reproducing as closely as possible the conditions used in the cryomicroscope. Film and developing conditions were identical: film from exactly the same batch was used in the two microscopes, and the doses were determined from densitometry in the same way. The published film speed was used to calculate the dose rates in absolute units.

Series of diffraction patterns were recorded as a function of electron dose from L-valine crystals mounted on various substrates. After rejecting some, a total of 21 series were analysed. Each of the diffraction patterns was examined to determine the maximum number of diffraction orders visible along the two axial directions, the corresponding spatial frequencies were calculated using the published lattice parameters for L-valine⁸ ($b = 5.27 \text{ \AA}$, $c = 9.71 \text{ \AA}$), and the average of the inverses of these two numbers, called R , was calculated to represent the maximum 'resolution' available in the pattern. The value of R is plotted as a function of dose, similar to a plot used earlier by Glaeser and Taylor⁹. It is perhaps more common to plot the reduction of intensity of certain spots and to quote the '1/e' value, but this is of greatest meaning when the fading of intensity is exponential (a straight line on a semilog plot), and this was

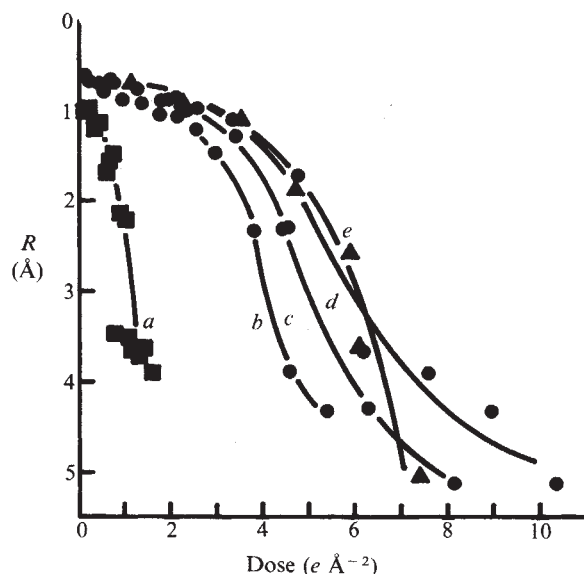


Fig. 1 Radiation damage in crystals of L-valine. The value of R indicates the maximum resolution remaining in the diffraction pattern: R is the inverse of the average spatial frequency corresponding to the highest diffraction orders visible in the two axial directions in the pattern. This is plotted as a function of the dose of electrons. The squares (curve *a*) represent data from three series of diffraction patterns recorded at room temperature using a Siemens 102 electron microscope at 60 kV. Circles (curves *b*, *c*, *d*) represent data from diffraction patterns recorded at liquid helium temperature at 65 kV using the cryomicroscope at Duke University. Triangles (curve *e*) represent data from two series of diffraction patterns recorded using the Siemens cryomicroscope in Berlin, which was operated at 60 kV. No adjustments have been made to the data to account for the small difference in voltage. Using the normal scaling, the inelastic scattering cross-section would be decreased by 7% going from 60 to 65 kV^{12,15}. Comparing curve *a* with the other curves illustrates that there is a reduction in radiation sensitivity of L-valine at low temperature, but the improvement is not the factor of ~70 that was proposed earlier. In the present experiment the differences between curves *b*–*e* are not considered significant, and further measurements will be needed to establish if there are useful differences between various substrates or operating conditions.

not usually seen in the low temperature L-valine diffraction pattern.

Selected results are presented in Fig. 1. The improvement due to the low temperature is given by the multiplying factor needed to scale the room temperature curve (*a*) up to the level of the liquid helium data. The improvement factor is in a range of 4–6. The use of high-conductivity substrates was supposed to allow a very great increase in the tolerance to radiation damage, but for all of the substrates tested the results were within this range. (The numerical average of 14 series recorded in our normal operating conditions with a variety of substrates produced an improvement factor of 5.) Because this degree of improvement is far below the reported values for L-valine of 67 or 87, we also checked the specimen temperature by providing extra liquid helium cooling to the thermal shields. The damage rate remained approximately the same as before. (The numerical average of five series recorded in these special operating conditions produced an improvement factor of about 6, but the data are insufficient to determine if the results of these conditions are significantly different from those of our normal conditions.)

The great difference between our results and the recently published values for L-valine prompted us to do a parallel experiment using the Siemens cryomicroscope at the Fritz-Haber-Institut in Berlin. The superconducting lens and cryostat of this microscope¹⁰ are similar to those used by Knappek and Dubochet, but the Berlin microscope allows operation at 60 kV for a direct comparison with our results at Duke University. We took to Berlin and used there the same developer and the film from the same batch that were used for our experiments at Duke. The results are shown in curve *e* of Fig. 1. It is clear that the rate of radiation damage to L-valine in the Berlin cryomicroscope is approximately the same as our previous results at Duke University.

There are differences between our results and those of Dietrich *et al.* and of Knappek and Dubochet, both at room temperature and liquid helium temperature. At room temperature, our L-valine diffraction patterns are of higher initial resolution. Curve *a* of Fig. 1 indicates a resolution better than 1 Å, while Dietrich *et al.* list a maximum resolution of 1.38 Å, and Knappek and Dubochet say that the resolution in L-valine is never better than 1 Å. Furthermore, the room temperature measurements of Heide *et al.*¹¹ for L-valine are generally consistent with our measurements and also disagree with those of Knappek and Dubochet. At liquid helium temperature, as in room temperature microscopy, drift may cause an apparent improvement in radiation tolerance if fresh areas of crystal are unknowingly illuminated by the beam. While specimen stages are quite stable in cryostats at thermal equilibrium, stage drift can occur after a temperature change (as when changing specimens, or when operating electrical devices that add heat to the cryostat.) A greater problem may be illumination drift, which we observed in the Berlin microscope. Although a selected area diffraction aperture is available in Berlin to compensate for this effect, there is no evidence that this was used by Knappek and Dubochet.

Our results therefore contradict the high 'protection factors' originally proposed by Dietrich *et al.* and Knappek and Dubochet. (Note here that J. Lepault and J. Dubochet, in presenting their latest results at the 10th International Congress on Electron Microscopy in Hamburg, now report 'protection factors' in L-valine that are similar to the values that we have found.) Nevertheless, the resolution of the pattern is higher at low temperature than at room temperature, as is shown in Fig. 1. Increased high-order spot intensities are due in part to the reduced Debye-Waller factor¹². A dose of $1 \text{ e \AA}^{-2}$ can be accumulated at liquid helium temperature before the L-valine diffraction pattern decays to the initial resolution at room temperature. For a given resolution, five times more electrons can be accumulated at liquid helium temperature than at room temperature, thus providing images with greater statistical significance. There is some preliminary evidence that a mercury

stain is significantly stabilized at low temperature¹³, possibly protected by a 'cage effect'¹⁴. This may partially explain a result of Dietrich *et al.*¹⁴, where a Pt-stained preparation showed a reduction of damage at low temperature: the observation may have reflected the stabilization of the platinum rather than the organic material. The effect of electron radiation on biological specimens at low temperature is clearly a complicated matter that warrants further careful study.

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Origin of the receptor potential in inner hair cells of the mammalian cochlea—evidence for Davis' theory

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The primary sensory hair cells of the mammalian cochlea are located in the organ of Corti, a sensory epithelium which separates fluids of widely differing chemical composition. The apical, sensory surfaces of the hair cells are exposed to the potassium-rich endolymph of the scala media and their lateral and ventral surfaces are exposed to the perilymph of the scala tympani whose chemical composition resembles that of other extracellular fluids¹⁻³. The high potassium concentration of the endolymph (150 mM) is believed to result from the activity of electrogenic potassium pumps located in the stria vascularis which lines the lateral walls of the cochlea³. These pumps are also the source of the positive endocochlear potential of about +80 mV which can be recorded from the scala media⁴⁻⁶. When the ear is stimulated with sound, receptor potentials may be recorded extracellularly from the fluid-filled spaces of the cochlea^{7,8}, and intracellularly from the rows of inner and outer hair cells⁹⁻¹¹. According to the 'resistance microphone' theory of Davis¹², these receptor potentials are derived from the pre-existing polarization of the hair cells by a change in the ohmic resistance of the mechanosensitive portion of the cell membrane (Fig. 1). This produces potential changes in the scala tympani and scala media of opposite phase, thus giving rise to the cochlear microphonic (CM). Evidence is presented here to support this theory. When sufficient depolarizing current is injected into inner hair cells to cancel the polarizing voltage, the receptor potentials disappear, and their phase is reversed when the polarizing voltage across the apical membranes of the hair cells is reversed.

The magnitude of the polarizing voltage is dependent on the size of the endocochlear potential, the hair cell resting potential and the identity of the ions carrying the receptor current³. It is generally accepted that potassium ions have an important role in transduction in the cochlea³; however, direct evidence that it carries the receptor current has only been obtained for hair cells of the frog sacculus¹³, although the channels are non-specific. If potassium ions carry the receptor current in cochlear hair cells, then the polarization voltage across the apical surface, needed to abolish the receptor potential, should equal the sum of the hair cell resting potential and the endocochlear potential, because the equilibrium potential for potassium across the apical membrane of cochlear hair cells is close to zero³.

The experiments were performed on guinea pigs, 180-300 g in weight, which were anaesthetized with pentobarbital sodium, Operidine and Droleptan (Janssen)¹⁴. The techniques used have been fully described elsewhere¹⁵. Gated tone bursts from a Beyer DT48 earphone were presented to the right ear through a closed sound system equipped with a probe microphone for monitoring the sound pressure at the eardrum. The envelope

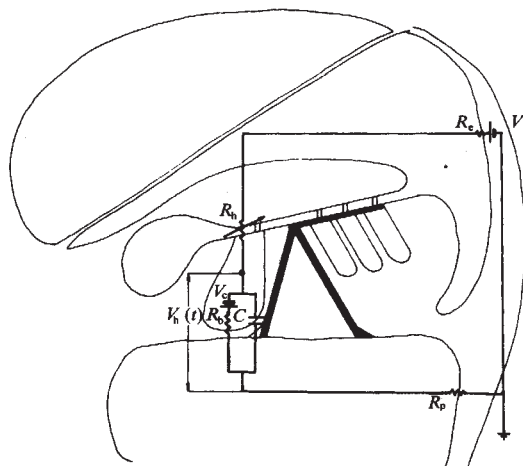


Fig. 1 Davis' resistance microphone theory, the membrane at the apex of the hair cell acts as a variable resistance (R_h), modulating the current driven through the cell by the positive endocochlear potential (V_e) and the negative hair cell resting potential (V_c). ($V_h(t)$) is the instantaneous voltage developed across the hair cell membrane. R_b and C are the resistance and capacitance of the basal and lateral membranes of the hair cell. R_e and R_p are resistances in the extracellular current pathways associated with the endolymph and perilymph respectively.

of the tone burst had a 2-ms risetime and a transient offset. The ventral surface of the basilar membrane in the basal turn of the cochlea was revealed through a small opening in the bony wall. The measurements reported here are from preparations in which the opening was made without a loss in sensitivity to the high frequencies (14-20 kHz) to which this part of the basilar membrane is tuned. Signals from the microelectrodes were fed to a high-input impedance preamplifier equipped with capacitance compensation and a bridge balancing circuit for current injection. The electrodes (100-130 M Ω impedance and filled with 3M KCl) were selected on their ability not to rectify when passing 1 nA of current into the perilymph of the scala tympani. With capacitance compensation, the bridge was adjusted to abolish the instantaneous voltage change associated with the injected current. This adjustment was made intracellularly and checked, after recording, in the fluid-filled compartments of the cochlea.

Intracellular recordings were made from inner hair cells which were identified as such on the basis of their electrophysiological characteristics¹⁵. They have small resting membrane potentials (-30 to -55 mV), large, asymmetrical receptor

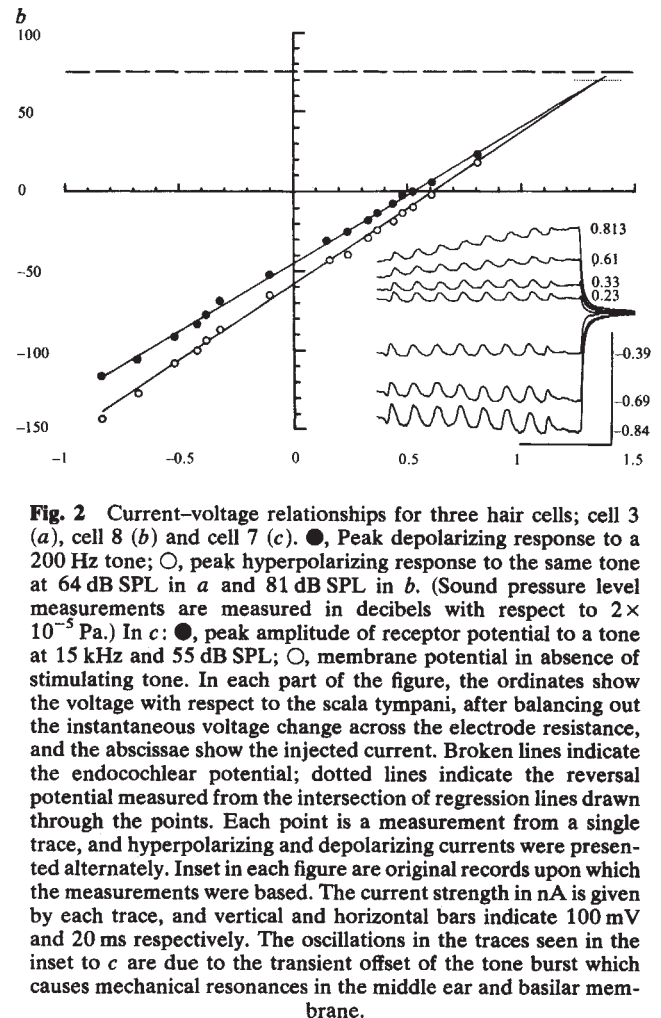
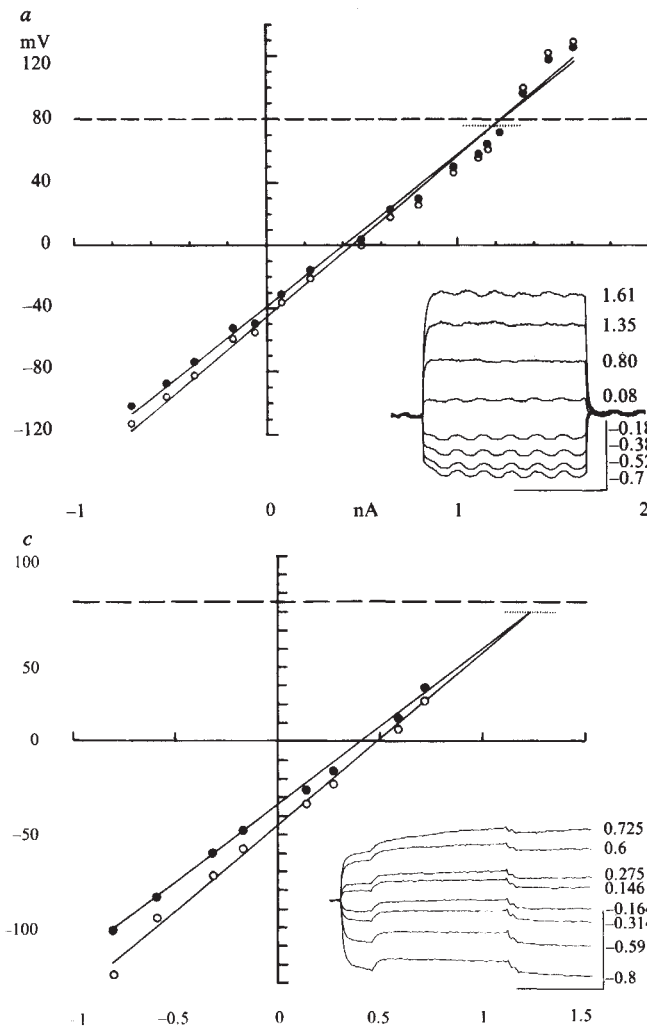


Fig. 2 Current-voltage relationships for three hair cells; cell 3 (a), cell 8 (b) and cell 7 (c). ●, Peak depolarizing response to a 200 Hz tone; ○, peak hyperpolarizing response to the same tone at 64 dB SPL in a and 81 dB SPL in b. (Sound pressure level measurements are measured in decibels with respect to 2×10^{-5} Pa.) In c: ●, peak amplitude of receptor potential to a tone at 15 kHz and 55 dB SPL; ○, membrane potential in absence of stimulating tone. In each part of the figure, the ordinates show the voltage with respect to the scala tympani, after balancing out the instantaneous voltage change across the electrode resistance, and the abscissae show the injected current. Broken lines indicate the endocochlear potential; dotted lines indicate the reversal potential measured from the intersection of regression lines drawn through the points. Each point is a measurement from a single trace, and hyperpolarizing and depolarizing currents were presented alternately. Inset in each figure are original records upon which the measurements were based. The current strength in nA is given by each trace, and vertical and horizontal bars indicate 100 mV and 20 ms respectively. The oscillations in the traces seen in the inset to c are due to the transient offset of the tone burst which causes mechanical resonances in the middle ear and basilar membrane.

potentials (15–35 mV peak to peak at 100 dB sound pressure level (SPL)) in response to low frequency tones of a few hundred Hertz (Fig. 1), and they generate large d.c. receptor potentials (15–24 mV at 60 dB SPL) in response to tones close to their characteristic frequencies (CF).

The polarizing voltage across the apical membrane of inner hair cells was changed by current injected through the intracellular recording electrode, and the relationships between the membrane potential and the injected current were plotted for the peak depolarizing and hyperpolarizing phases of the receptor potential of the hair cells in response to tones at 200 Hz, and for the resting potential and d.c. receptor potential in response to tones at 15 kHz (Fig. 1).

In two of the cells (numbers 3 and 6) it was possible to deliver sufficient current to reverse their low frequency receptor potentials (Fig. 1a). The peak-to-peak amplitudes of the receptor potentials were 5 mV (3) and 15 mV (6) for 200 Hz tones at 64 and 93 dB SPL respectively. The reversal potentials of these hair cells, +80 mV (3) and +60 mV (6) respectively, coincided with the endocochlear potential, which was measured from the scala media by advancing the recording electrode past the inner hair cells after making the intracellular recordings (Table 1).

In a further six inner hair cells it was not possible to make direct measurements of the reversal of the receptor potentials because the recording system rectified strongly in the depolarizing direction. The current-voltage relationships of the hair cells were relatively linear over the range +40 mV to -150 mV (Fig. 2b, c) and it was possible to estimate the reversal of the receptor potentials recorded in response to low (Fig. 2b) and high frequency tones (Fig. 2c) by extrapolating the two regression lines drawn through the measured peak hyperpolarizing and depolarizing phases of the receptor potential. It was discovered,

for each hair cell, that the intersection of these lines occurred at a potential close to the endocochlear potential (Table 1).

These results are an indication that potassium ions carry the receptor current in inner hair cells and they show that the selectivity of the transducer channels is similar to those of hair cells in the frog sacculus¹³ and turtle cochlea¹⁶, whose receptor potentials also reverse close to the endolymphatic potential (which is close to zero in these preparations). Furthermore the results support Davis' model of transduction in the cochlea, at least in relation to the function of inner hair cells, and they are consistent with previous measurement which showed that the amplitude of the d.c. component of the receptor potential, and the resistance change associated with it, are linearly related to each other¹⁵.

The current-voltage relationships of all the inner hair cells examined were linear over a large range without the strong

Table 1 A comparison of the reversal potentials of inner hair cells and the endocochlear potential measured with respect to scala tympani

Cell no	Resting potential (mV)	Input resistance (mΩ)	Reversal potential (mV)	Endocochlear potential (mV)
1	-55	76	83	79
2	-50	99	85	81
3	-45	90	75	80
4	-30	79	85	90
5	-45	78	90	85
6	-32	89	63	60*
7	-45	93	73	85
8	-55	92	70	75

* This low value of endocochlear potential was probably brought about by an interruption in the guinea pigs' respiration during surgery.

outward rectification seen in cells in the turtle cochlea¹⁶ and frog sacculus¹³. It must be presumed that the nonlinear responses of the cochlear microphonics and summing potential to the injection of current into the scala media, are due to voltage-dependent electrical or mechanical components in the cochlea, possibly associated with the outer hair cells¹⁷⁻²¹.

From the slopes of the current-voltage relationships, it was possible to measure the input resistance, its inverse, the leakage conductance of the hair cells, and the increase and decrease of the transduction current associated with peak, saturating, depolarizing and hyperpolarizing receptor potentials, respectively. These measurements were made in five cells whose maximum peak-to-peak receptor potentials at 200 Hz ranged over 15–30 mV. The amplitude of the depolarizing phase of the receptor potential is four times that of the hyperpolarizing phase and this is reflected in the difference in their slope conductances. The mean leakage conductance of the five hair cells is 10.7 ± 3.2 nS, the total change in the transduction conductance is 1.1 ± 0.5 nS the increase associated with the

depolarizing phase is 0.85 ± 0.35 nS and the decrease associated with hyperpolarization is 0.25 ± 0.15 nS. The peak transducer conductance measured in inner hair cells is similar to that measured in turtle cochlear hair cells¹⁶ and hair cells of the frog sacculus¹³, which are 4.08×10^{-9} S and 2.5 nS respectively. The transducer channels in saccular hair cells are similar in size to the acetylcholine channel, thus it might be expected that they have the same elementary conductance of 25 pS²². The transducer channels are believed to be located at the tips of the stereocilia in sacculus hair cells²³, of which there are ~100, that is, one channel per stereocilium²⁴. If the transducer channels of inner hair cells share these properties, then there are about 40 channels controlled by the stimulus. Each inner hair cell in the guinea pig cochlea bears between 34–40 stereocilia²⁵ which also represents a ratio of one channel per stereocilium.

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Abnormal visual input leads to development of abnormal axon trajectories in frogs

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Throughout the normal vertebrate brain, visual maps from the left and right eyes overlap and are in register with one another. Visual input has a major role in the development of the pathways which mediate these binocular projections¹⁻³. A dramatic example of the developmental role of sensory input occurs in the isthmo-tectal projection, which is part of the polysynaptic relay from the eye to the ipsilateral tectum of the frog, *Xenopus laevis*⁴⁻⁸. If one eye is rotated when the animal is still a tadpole, the isthmic axons respond by changing the topography of their terminations in the tectum; for example, a given isthmo-tectal axon which normally would connect with medial tectum can be induced to terminate in lateral tectum. Such rearrangements bring the ipsilateral visual map into register with the contralateral retinotectal map, even though one eye has been rotated. Indirect evidence⁹ has suggested that after early eye rotation, isthmo-tectal axons do not grow directly to their new tectal targets but instead reach those targets by routes which pass through their normal termination zones. Here I have used anterograde horseradish peroxidase labelling of isthmo-tectal fibres to show the trajectories of such axons and to compare them with the routes which axons take when allowed to develop normally. Tracings of individual axons in flat-mounted, unsectioned tecta show that most axons in normal *Xenopus* follow fairly straight paths in the tectum. In contrast, early eye rotation causes many isthmo-tectal axons to follow crooked, circuitous pathways before they terminate.

To fill isthmo-tectal axons, horseradish peroxidase (HRP) was injected into the nucleus isthmi (NI) of seven normal and eight eye-rotated frogs. (Unilateral eye rotation had been performed at stages 52–55 of Nieuwkoop and Faber⁹, when NI

Table 1 Numbers of axons crossing ml and rc lines

Animal	ml crossings	rc crossings	Crossing index ($\frac{ml - rc}{ml + rc}$)
Normal			
N1	101	69	0.19
N2	148	71	0.35
N3	159	59	0.46
N4	299	147	0.34
N5	46	25	0.30
N6	387	154	0.43
N7	240	129	0.30
Total	1,380	654	
Eye-rotated			
R1	79	51	0.22
R2	332	232	0.18
R3	38	32	0.08
R4	193	160	0.09
R5	13	12	0.04
R6	64	67	-0.02
R7	220	222	0.00
R8	164	154	0.03
Total	1,103	930	

cells are still being formed^{10,11} and before the appearance of an electrophysiologically detectable ipsilateral visuotectal map¹².) After 3 days, the contralateral tectum was removed, flattened, fixed by immersion in glutaraldehyde and reacted for visualization of the enzyme¹³. As isthmo-tectal axons run in superficial laminae and are essentially parallel to the tectal surface, the whole course of an isthmic axon can be followed from the point of entry into the tectum to the site(s) of termination. Figure 1a shows that axons in normal *Xenopus* follow a route which is approximately rostrocaudal; there is some criss-crossing but the tendency is for axons to run parallel to one another. Some axons zig-zag, and some have branches which diverge but later reconverge and terminate together (Fig. 1b–e).

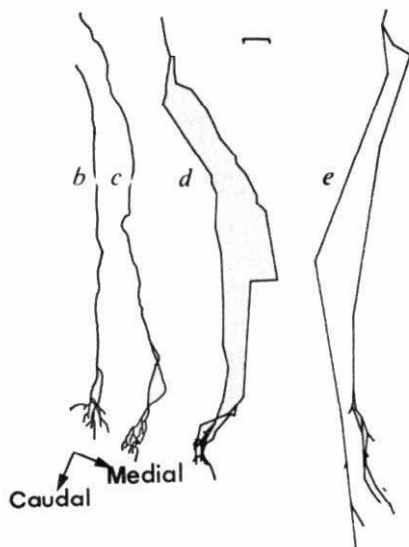
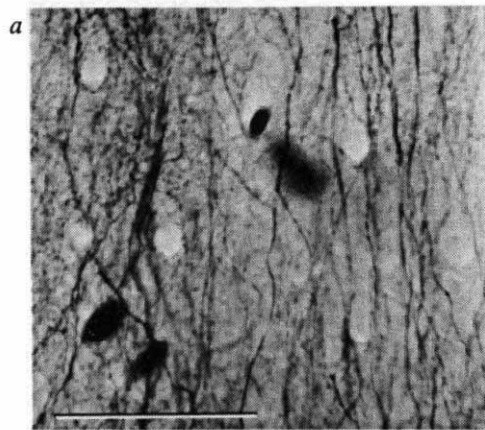


Fig. 1 *a*, Low-power view of filled axons. Dark round and oval spots are red blood cells. *b-e*, Four filled axons: *b* and *c* are camera lucida drawings; *d* and *e* are computer reconstructions. All scale bars, 100 μ m.

Methods: 5–15 nl of ~30% HRP (Sigma type VI) with 1% lysolecithin²⁰ was injected using pressure into the right NI. After 3 days survival, the left tectum was exposed, fixed for 2 min with 2.5% glutaraldehyde in phosphate buffer, and flattened between a coverslip and slide; after 2 h, the tecta were rinsed in buffer, left overnight at 4 °C in 0.05% diaminobenzidine, 0.025% cobalt chloride and 0.02% nickel ammonium sulphate in 0.1 M phosphate buffer at pH 7.3 (ref. 10); the next day fresh mixture plus H₂O₂ was added for 1–2 h.

A different pattern was expected in *Xenopus* with one eye rotated at mid-larval stages. As Fig. 2 shows schematically, 180° rotation of one eye can induce an isthmo-tectal axon to terminate in lateral rather than medial tectum and vice versa. (Comparable rearrangements also occur in response to smaller degrees of rotation^{8,14}.) Previous studies had suggested that these axons reach their new locations only after passing through their normal locations⁸. For example, an axon might grow to medial tectum and then be rerouted laterally. The HRP-filled axons in eye-rotated *Xenopus* are consistent with this prediction. Figure 3 shows axons crossing one another and running in medial and lateral directions as well as rostrocaudally. Moreover, some appear to arborize in more than one place and some reverse directions (Fig. 3*b-d*).

To quantify the differences between trajectories in normal and eye-rotated tecta, a numerical measure was devised. If a pair of orthogonal lines is superimposed on a group of essentially parallel axons, running rostrocaudally as shown in Fig. 4*a*, then all axons cross one line ('ml') and none or one cross

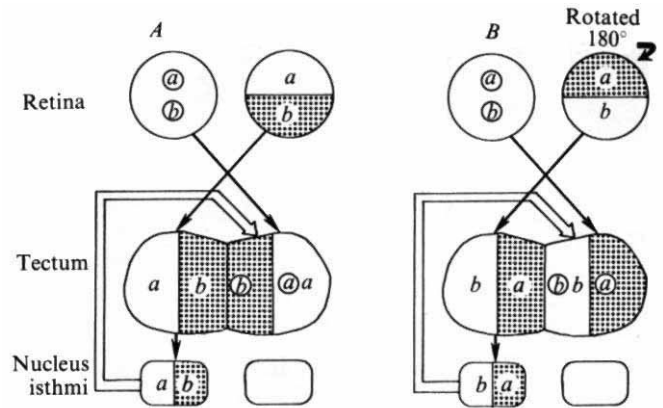


Fig. 2. Schematic explanation of topographic rearrangements after 180° rotation of right eye. *A*, normal topography. *a*, *b*, Positions in inferior and superior visual field. Right inferior retina (shaded) projects via left medial tectum to left medial NI, which projects to right medial tectum. This pathway brings input for visual field *b* as seen by the right eye to the part of the tectum which receives corresponding input from the left eye. *B*, right eye is rotated 180°, so that embryonically inferior retina (shaded) now sees visual field *a*, not *b*. It connects normally to medial tectum, which projects to left medial NI. Medial NI now connects to right lateral tectum, bringing visual field *a* into register with visual field *a* from the left eye. Comparable rearrangements occur in the projection from right NI to left tectum (not shown).

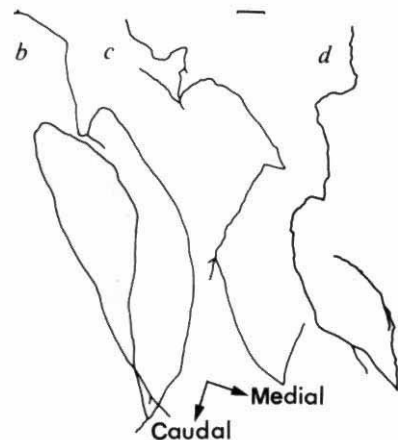
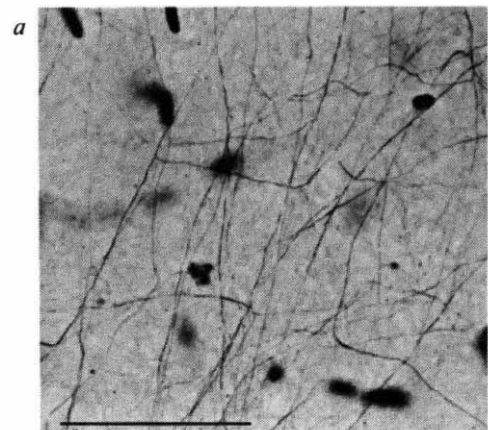


Fig. 3 HRP-filled isthmo-tectal axons in eye-rotated *Xenopus*. *a*, Low-power view. *b-d*, Camera lucida drawings. Collaterals were visible but no terminal arbors were filled in these axons. Scale bar, 100 μ m.

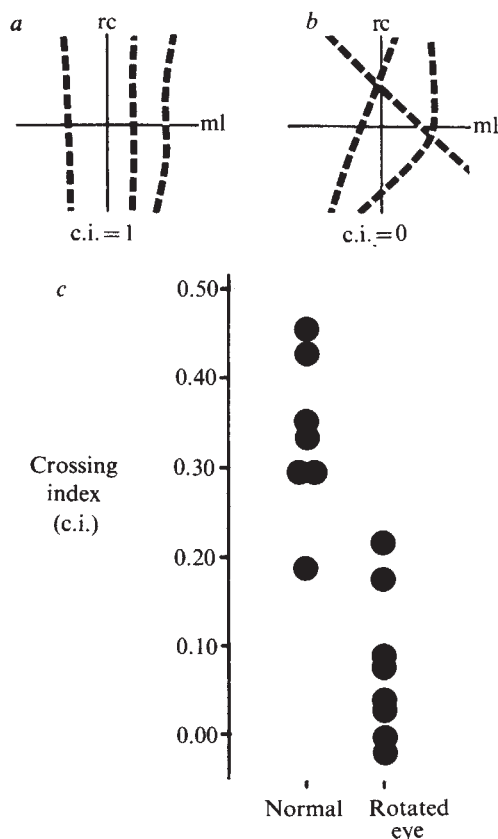


Fig. 4 *a*, Schematic view of axons (dashed lines) crossing mediolateral and rostrocaudal lines, showing examples of essentially parallel axons. Crossing index = 1.0. *b*, Crossings by randomly oriented axons. Crossing index = 0.0. *c*, Crossing indices in tecta of seven normal (left) and eight eye-rotated (right) *Xenopus*. Each circle represents one tectum.

the other line ('rc'). Randomly oriented axons would on the average cross both lines in equal numbers (Fig. 4*b*). For each of the animals included in this study, a grid of rostrocaudal and mediolateral lines, 1 inch apart, was superimposed on an $\times 85$ photograph of the tectum. The area around each grid intersection was surveyed for the presence of filled axons. There were 6 to 21 suitable areas per tectum. Using a camera lucida, the images of the filled axons at a magnification of 450 were superimposed on 3-inch segments of the intersecting lines. The number of axons which crossed each line was counted for each region. (For example, in Fig. 4*b*, both lines are crossed three times.) The crossing index $ml - rc / ml + rc$ was calculated. Perfect rostrocaudal orientation would give a value of 1.0; other orientations and less orderly routes would yield smaller values, and random order would give a value of 0.0. Figure 4*c* and Table 1 show the crossing indices for tecta of normal and eye-rotated animals. They are different at the 0.01 level using a Mann-Whitney *U*-test¹⁵. This result is consistent with the subjective assessment that isthmo-tectal axons in eye-rotated animals follow much less orderly routes than do normal isthmo-tectal axons.

Other studies of the tectum of amphibians and goldfish have also demonstrated that axons can take circuitous routes to their final destinations. These studies have focused primarily on the trajectories of regenerating optic axons (refs 16–19 and R. M. Gaze and J. Fawcett, personal communication), and they demonstrate that a regenerating optic axon which begins growing along an unsuitable pathway can change course to reach its proper termination site. In contrast, developing isthmo-tectal axons in eye-rotated *Xenopus* begin their routes normally but end their growth in different positions from normal. Moreover, visual input does not influence the orientation of the visual map that regenerated optic axons form, whereas visual input is the

critical determinant of the orientation of the visual map of the isthmo-tectal projection¹⁴.

In conclusion, both qualitative and quantitative assessments show that abnormal visual input resulting from early eye rotation can produce abnormal trajectories in isthmo-tectal axons. This result suggests that the axons are initially guided by non-visual cues to approximately appropriate sites but that mismatched visual inputs from the two eyes either promote renewed growth or allow maintenance of branches which normally would have been withdrawn.

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Dopamine modulates cholecystokinin release in neostriatum

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Peptides of the cholecystokinin family, but mainly the sulphated octapeptide (CCK₈), have been found in brain extracts of several species^{1–6}. High amounts are present in axons and nerve endings in the rat neostriatum (caudate-putamen)^{7,8} and a role for cholecystokinin as a neurotransmitter in this functionally important area is possible^{9,10}. We have incubated slices of rat caudate-putamen (CP) to study the release of cholecystokinin-immunoreactivity (CCK-IR) *in vitro*. The release of CCK-IR was induced by veratridine. It was dependent on the presence of Ca²⁺ in the incubation medium and was blocked by tetrodotoxin. We now present evidence that dopaminergic agonists added to the slices modulate the veratridine-induced release via different groups of receptors. Receptors which mediate an enhancement of the release of CCK-IR seem to be located on afferent axons and nerve endings and are possibly of the D-2 subtype. Receptors which mediate an attenuation of the release are probably situated on cells intrinsic to the CP. These receptors seem to be coupled to adenylate cyclase and might thus be of the D-1 subtype. There is also evidence that endogenous dopamine when released enhances the secretion of CCK-IR.

Cholecystokinin is found only in axons and nerve endings in the CP of rat brain^{7,8}, whereas the respective perikarya are located in the claustrum and/or the piriform cortex and the amygdala¹¹. *In vitro* preparations of the CP are thus ideally suited for investigating the presynaptic regulation of cholecys-

tokinin release. Horizontal slices (0.3 mm thick) of the rat CP were prepared with a McIlwain chopper. Only the head of the CP, a region in which cholecystokinin is evenly distributed (unpublished results), was used. The slices were incubated in tubes and the cholecystokinin released was determined by radioimmunoassay. When added to the slices in amounts of 25, 50 and 100 pg, respectively, CCK₈ could be recovered without loss after a 20-min incubation period (recovery ranged over 95–103%; for further information on the radioimmunoassay and details of the methods see Fig. 1 legend).

Veratridine stimulated the release of CCK-IR in a concentration-dependent manner, showing maximal effect at 7.5×10^{-6} M (Fig. 1). During the incubation period, approximately 5% of the tissue content (150 pg per mg wet weight) of CCK-IR were released. This immunoreactivity was resistant to boiling and thus was not due to contamination with peptidases. The release of CCK-IR induced by veratridine (7.5×10^{-6} M) was reduced by tetrodotoxin (5×10^{-7} M) to 0.02% ($P < 0.001$) of that seen in controls. In medium devoid of Ca^{2+} , veratridine released only 0.02% of the amount found in control medium ($P < 0.001$).

Veratridine (5×10^{-6} M) was used for all further experiments. The dopaminergic agonists apomorphine and dopamine had no effect on the basal release of CCK-IR (data not shown), but

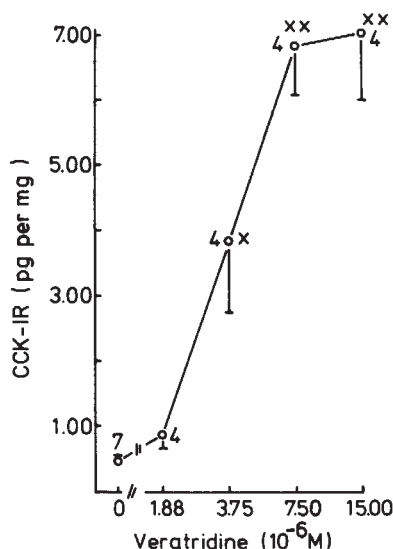


Fig. 1 Effect of veratridine on the release of CCK-IR from slices of rat caudate-putamen. Ordinate: CCK-IR release is expressed as pg per mg wet weight over a period of 20 min; abscissa: concentration of veratridine. $\bar{x} \pm \text{s.e.m.}$ (n). In these and the following experiments, statistical analysis was performed with one-way ANOVA and Scheffe's test. $\times$, $P < 0.05$; $\times\times$, $P < 0.01$ as compared with release without veratridine.

Methods: Male Wistar rats (200–250 g) were decapitated and their brains quickly removed and placed on a cooled plate (0°C). The head of the CP was dissected by two coronal cuts and the nucleus accumbens and any cortical tissue, especially the claustrum, were carefully removed. Horizontal slices (0.3 mm) were cut, pooled, incubated for 60 min and then distributed into plastic tubes (two or three slices per tube, ~10–15 mg wet weight). Within one experiment tissue weights did not vary by more than 30%. The incubation medium was collected every 20 min for CCK assay. During the second and third collection periods there was a stable basal release of CCK-IR. At the beginning of the fourth period veratridine was added to a few tubes and was present for 20 min. After this period the slices were blotted and weighed. The incubation medium was composed of (mM): NaCl 118, KCl 4.8, CaCl_2 1.3, MgSO_4 1.2, NaHCO_3 25, KH_2PO_4 1.2, glucose 11, ascorbic acid 0.11, bacitracin 0.02; bovine serum albumin (0.1%). It was bubbled with a mixture of CO_2 5%/O₂ 95%. CCK-IR was determined by radioimmunoassay as described previously⁶. The antiserum (a gift of M. C. Beinfeld) is also able to recognize CCK peptides which are shortened at the N-terminal, that is, possible metabolites of CCK which might be produced despite the presence of the peptidase inhibitor bacitracin. CCK₃₃ (a gift of V. Mutt) was iodinated and used as label, CCK₈ was used as standard.

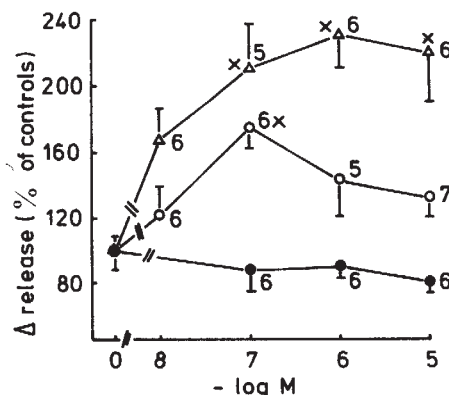


Fig. 2 Effect of dopamine on the release of CCK-IR induced by veratridine alone (○) or following kainic acid injection (Δ) or in the presence of IBMX (●), from slices of rat caudate-putamen. $\bar{x} \pm \text{s.e.m.}$ (n). $\times$, $P < 0.05$ as compared with incubations with veratridine alone.

Methods: The slices were incubated as described in Fig. 1 legend. Veratridine (5×10^{-6} M) was present during the fourth collection period, whereas all other drugs were first added at the beginning of the third period. These drugs did not affect basal release. The ordinate shows release in per cent of controls. For these calculations the basal release of the third period was subtracted from the stimulated release of the fourth period. Within one experiment the mean of the net releases of CCK-IR induced by veratridine alone was taken as 100%. The abscissa shows the concentration of dopamine used. In studies on the effect of dopamine in control tissue, veratridine alone released 5.52 ± 0.67 pg per mg wet weight ($n = 9$). The effect of dopamine on release of CCK-IR from slices of CP was also tested 5 days after unilateral injections of kainic acid ($1.5 \mu\text{g}$ per $1.5 \mu\text{l}$). Veratridine alone released 11.46 ± 3.00 pg per mg ($n = 10$) from slices of the injected side and 8.09 ± 1.60 ($n = 6$) from slices of the contralateral intact side. The injection had the following parameters: 9.2 mm anterior to the i.a.-line, 2.5 mm lateral to the midline, 6.0 mm ventral to the brain surface, plane angle 5° down. In studies on the effect of dopamine on CCK-IR release in the presence of IBMX (10^{-3} M), veratridine alone released 6.93 ± 1.15 pg per mg. IBMX is known to antagonize the effects of adenosine, which may be a neurotransmitter in the brain²¹. However, adenosine (10^{-6} – 10^{-4} M) had no effect on the veratridine-induced release of CCK-IR (manuscript in preparation).

affected veratridine-induced release in a complex concentration-dependent manner. Small concentrations of the agonists (dopamine 10^{-7} M or apomorphine 10^{-8} M) enhanced the release; higher concentrations, however, had no significant effect (Figs 2, 3). To explain this phenomenon we assumed that the agonists caused their effects by acting on distinct groups of receptors with stimulatory and inhibitory properties, respectively.

Kainic acid was used to investigate these receptors further. The agent is known to destroy perikarya, but to leave afferent axons and nerve endings intact^{12,13}. Five days after unilateral injections of kainic acid into the CP of rats, the higher concentrations of dopamine (10^{-6} and 10^{-5} M) also significantly increased veratridine-induced release of CCK-IR (Fig. 2). Thus, only the facilitatory receptors had survived the treatment with kainic acid. They seem to be situated on neuronal afferents and might thus belong to the D-2 subtype^{14,15}. In support of our assumption haloperidol (10^{-7} M), a dopaminergic antagonist with a preference for D-2 receptors, abolished the dopamine-induced enhancement of the release of CCK-IR (Table 1a). These receptors might be located on the CCK terminals. It is also possible that they affect the release of another transmitter which in turns increases the secretion of CCK-IR.

Because kainic acid treatment abolished the inhibitory component of the action of dopamine on CCK-IR release, neurones intrinsic to the CP must mediate this effect. The dopamine receptors involved might be of the D-1 subtype^{14,15}. However, the lack of availability of specific D-1-receptor antagonists means that this cannot be tested directly, and so an indirect

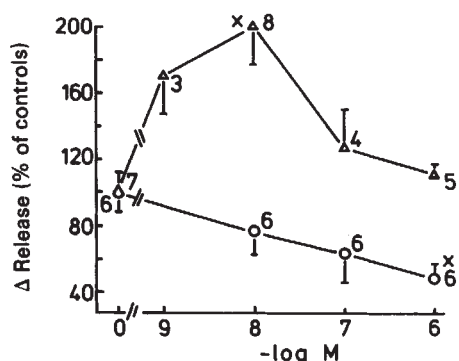


Fig. 3 Effect of apomorphine (Δ) and haloperidol ($\circ$) on the release of CCK-IR induced by veratridine. $\bar{x} \pm \text{s.e.m.}$ (n). $\times$, $P < 0.05$ as compared with controls.

Methods: Slices of CP were incubated as described in Fig. 1 legend. Apomorphine and haloperidol were first added at the beginning of the third collection period. Neither agent affected basal release. The ordinate shows release in per cent of controls (as described for Fig. 2). Control release was 8.60 ± 0.93 pg per mg wet weight ($n = 6$) in the experiments with haloperidol and 6.58 ± 1.60 pg per mg ($n = 6$) in the experiment with apomorphine. The abscissa shows concentrations of drugs.

Table 1 CCK-IR release from rat caudate-putamen

		CCK-IR released (pg per mg wet weight)
<i>a</i>		
VE		$4.39 \pm 0.56(5)$
VE + DA		$6.98 \pm 0.75(5)^*$
VE + HP		$1.96 \pm 0.70(5)^*$
VE + DA + HP		$3.99 \pm 1.00(5)^\dagger$
<i>b</i>		
VE		$11.50 \pm 1.80(6)$
VE + HP	i.l.	$10.56 \pm 2.10(6)$
VE		$10.30 \pm 1.38(6)$
VE + HP	c.l.	$4.34 \pm 0.90(6)^*$

a, Antagonism of the dopamine (DA, 10^{-7} M)-induced enhancement of the release of CCK-IR by haloperidol (HP, 10^{-7} M). Release was stimulated by veratridine (VE, 5×10^{-6} M). * Indicates significant difference ($P < 0.05$) of release from slices treated with veratridine alone; $\dagger$ indicates significant difference ($P < 0.05$) of slices treated with veratridine plus dopamine. *b*, Effect of severance of the nigrostriatal pathway on the decrease in the release of CCK-IR by haloperidol (HP, 10^{-6} M). Release was stimulated by veratridine (VE, 5×10^{-6} M). A unilateral knife-cut was performed 7 days before the release experiment (2 mm blade, 4.3 anterior to the i.a. line, down to bone, medial edge in midline, plane angle 11° down). i.l., Ipsilateral; c.l., contralateral side. * Indicates significant difference from slices treated with veratridine.

approach was used^{15,16} D-1 receptors are known to be coupled to adenylate cyclase¹⁷⁻¹⁹. The increase in cyclic AMP which results from their stimulation can be enhanced by phosphodiesterase inhibitors such as 3-isobutyl-1-methylxanthine (IBMX)²⁰. If the dopamine receptors which inhibit the release of CCK-IR are indeed of the D-1 subtype, the effect of their stimulation should be increased by IBMX. The inhibitory effect of dopamine should now more strongly antagonize its facilitatory effect on the release of CCK-IR. In the presence of IBMX (10^{-3} M) all concentrations of dopamine tested failed to enhance the release of CCK-IR (Fig. 2), a result which is consistent with our assumption that the inhibitory receptors might be of the D-1 subtype. We do not know on which neurones these dopamine receptors are located or which transmitters are released by their stimulation.

Due to its mechanism of action, it was obvious that veratridine released not only CCK-IR, but also other substances including dopamine. Next, we investigated whether this endogenous dopamine modulated the release of CCK-IR. IBMX (10^{-3} M), when given alone, did not reduce the effect of veratridine on

CCK-IR release (it was 6.93 ± 1.15 and 7.22 ± 1.30 pg per mg wet weight in the absence and presence of IBMX, respectively; $n = 6$ for both groups). Thus, the concentrations of the released endogenous dopamine were not high enough to stimulate the inhibitory receptors effectively. Haloperidol, however, which preferentially antagonizes D₂ receptors, reduced the veratridine-induced release of CCK-IR in a concentration-dependent manner (Fig. 3). Seven days after unilateral surgical severance of the nigrostriatal pathway, that is, depletion of the CP of dopamine¹¹, haloperidol no longer had any effect on the release of CCK-IR (Table 1*b*). In contrast, it still significantly reduced the release of CCK-IR from slices of the contralateral intact side. Thus, haloperidol did indeed decrease CCK-IR release by antagonizing the effect of endogenous dopamine on facilitatory receptors.

It remains an open question whether endogenous dopamine modulates the release of cholecystokinin from the CP *in vivo* also. The CCK afferents of the CP link this structure with the limbic system and olfactory areas¹¹. Our data indicate that dopaminergic neurones modulate these inputs. Future work must focus on the relevance of these findings to the function of the basal ganglia in normal and pathological conditions.

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Immunoglobulin heavy-chain switching in pre-B leukaemias

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Immunoglobulin gene expression is initiated in pre-B cells by rearrangements of heavy-chain variable genes V, D and J, for transcription together with the constant region gene C_μ (refs 1-7). The subsequent joining of light-chain V-J genes in the κ or λ families^{8,9} leads to formation of complete IgM molecules, which are then expressed on the surface of B cells. Heavy-chain isotype switching has been thought to occur later and to involve C_H gene rearrangement via deletion of DNA between a switch site 5' to C_μ and a switch site 5' to the downstream C_γ, C_ε or C_α gene expressed by a mature plasma cell¹⁰⁻¹⁵. On the other hand, isotype switching has been seen in human pre-B-cell

leukaemias^{16,17} and in a murine pre-B-cell line before light-chain gene rearrangements and without C_{μ} gene deletion^{18,19}. To explore further the isotype switching in pre-B cells, we used monoclonal antibodies in immunofluorescence assays to allow unambiguous assignment of the heavy-chain isotypes expressed by individual leukaemic cells in 11 pre-B-cell leukaemias. Switching in these leukaemic clones invariably led to expression of $\gamma 1$ heavy-chain and κ light-chain determinants, occasionally together with $\gamma 4$ and α . These results indicate a nonrandom order for heavy-chain isotype switching and for light-chain isotype expression, and also suggest that a mechanism exists for coordinating the two events.

The diagnosis of pre-B-cell leukaemia was based on the morphology, terminal deoxynucleotidyl transferase activity, absence of T-cell and myeloid-cell markers, lack of surface immunoglobulin (sIg) and presence of cytoplasmic immunoglobulin (cIg) determinants in acute lymphocytic leukaemia cells of blood and bone marrow samples from affected children^{16,17}. The 11 examples studied here were selected from approximately 30 cases of pre-B-cell leukaemia on the basis that subpopulations of the leukaemic cells exhibited a non- μ heavy-chain isotype. The monoclonal antibodies with heavy and light chain specificities have been described elsewhere²⁰⁻²².

The frequencies of leukaemic cells with conventional pre-B phenotype, sIg $^{-}$ c μ^{+} , among bone marrow or blood mononuclear cells varied from >75% in six cases to <15% in the other five (Table 1). In all 11 patients we observed cells of pre-B phenotype which had intracytoplasmic $\gamma 1$ determinants; the frequencies of sIg $^{-}$ c $\gamma 1^{+}$ cells were 2–20%. In six cases, sIg $^{-}$ c $\gamma 4^{+}$ cells were found in very low frequencies (0.1–2%). One sample also contained a subpopulation of α^{+} pre-B cells, but none contained pre-B cells with intracytoplasmic δ , $\gamma 2$, $\gamma 3$ or ϵ determinants.

The c $\gamma 1^{+}$, c $\gamma 4^{+}$ and c α^{+} cells were quite distinct from plasma cells or plasmablasts with regard to their lack of detectable surface immunoglobulins, scanty cytoplasm with a large convoluted nucleus, less intense immunofluorescent staining for immunoglobulin determinants and their expression of terminal deoxynucleotidyl transferase, a marker of immature lymphoid cells. The staining intensity of these pre-B cells was slightly stronger and the immunofluorescence pattern was more homogeneous than that of c μ^{+} pre-B cells (Fig. 1).

In all 11 patients, variable proportions of the leukaemic cells expressed intracytoplasmic κ chains without detectable surface immunoglobulins (sIg $^{-}$ c κ^{+}). No c λ^{+} pre-B cells were detected. The frequencies of the c κ^{+} pre-B cells closely paralleled those

of the c $\gamma 1^{+}$ cells, but not the c μ^{+} pre-B cell frequencies (Table 1). Synchronous expression of $\gamma 1$ heavy chains and κ light chains was confirmed by two-colour immunofluorescence analysis (Table 2). The κ/λ ratio of sIg $^{+}$ B cells in these patient samples was within the normal range (1.5 ± 0.4), serum immunoglobulin levels were normal and no monoclonal IgM, IgG or IgA paraproteins were present in the serum (data not shown); these results indicate that few, if any, of the exclusively κ^{+} leukaemia cells matured beyond the pre-B cell stage.

These results confirm that heavy-chain isotype switching is a relatively frequent occurrence in leukaemic pre-B cells. Since these cells lack surface immunoglobulins, these data favour a stochastic model for isotype switching rather than an antigen-induced switch mechanism governed by T cells (see ref. 23). The relative frequencies of isotype switches, μ to $\gamma 1 > \gamma 4 \gg \alpha$ and the absence of δ , $\gamma 2$, $\gamma 3$ and ϵ indicate a preferential order for the switching process in leukaemic pre-B cell clones. One factor that potentially could influence the order of switching is the sequence of the C_H genes which are located on chromosome 14 (ref. 24). In mice, where the order of the C_H genes is μ , δ , $\gamma 3$, $\gamma 1$, $\gamma 2b$, $\gamma 2a$, ϵ and α ²⁵, it has been suggested that the location of the $C_{\gamma 3}$ gene, and/or a relatively high degree of complementarity between the switch sites 5' to C_{μ} and to $C_{\gamma 3}$ (ref. 23), favour the μ to $\gamma 3$ switch over those from μ to other γ isotypes²⁶. So far, the order of human C_H genes has only been partially elucidated. $C_{\gamma 2}$ is 5' to $C_{\gamma 4}$ (refs 27, 28); $C_{\gamma 1}$ appears to be 5' to $C_{\gamma 3}$ (ref. 28); and C_{ϵ} genes are thought to

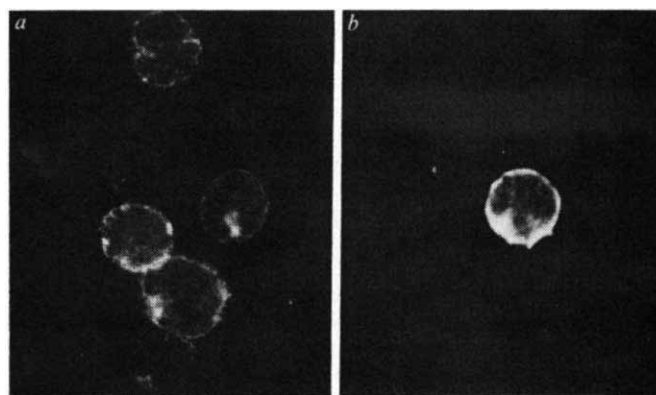


Fig. 1 Immunofluorescence staining patterns of leukaemia: a, c μ^{+} pre-B cells; b, c $\gamma 1^{+}$ pre-B cells; these lack detectable surface immunoglobulin.

Table 1 Expression of immunoglobulin isotypes by human pre-B leukaemia cells

Patient	Sample	Frequencies (%) of surface Ig $^{-}$ lymphoid cells that express cytoplasmic									
		μ	δ	$\gamma 1$	$\gamma 2$	$\gamma 3$	$\gamma 4$	α	ϵ	κ	λ
C.Pr.	PB	96.0	—	2.7	—	—	—	—	—	2.7	—
A.C.	PB	92.7	—	2.0	—	—	0.1	—	—	1.6	—
C.Ph.	BM	90.1	—	20.6	—	—	1.3	6.2	—	20.4	—
P.T.	BM	86.8	—	11.9	—	—	—	—	—	11.9	—
M.K.	BM	81.0	—	6.4	—	—	1.9	—	—	6.8	—
A.B.	BM	78.0	—	9.3	—	—	2.3	—	—	9.1	—
C.R.	BM	14.5	—	20.8	—	—	—	—	ND	20.6	—
K.K.	PB	4.9	—	3.5	—	—	—	—	ND	3.5	—
P.A.	BM	0.7	—	18.8	—	—	1.5	—	—	18.8	—
E.F.	BM	0.5	—	14.1	—	—	—	—	ND	13.4	—
P.K.	BM	0.3	—	3.8	—	—	0.5	—	—	3.6	—

Two-colour immunofluorescence analysis of surface and cytoplasmic immunoglobulin determinants¹⁰ was conducted by (1) staining viable peripheral blood (PB) or bone marrow (BM) mononuclear cells with fluorescein-labelled, affinity-purified goat antibodies to human immunoglobulin and, after washing and fixation of the cells, sedimented onto glass slides, (2) counter-staining for cytoplasmic components with mouse monoclonal antibodies specific for human immunoglobulin isotypes; rhodamine-labelled, affinity-purified goat antibodies to mouse immunoglobulin, which had been extensively absorbed with human immunoglobulins, were used to detect bound mouse monoclonal antibodies. The monoclonal anti- μ (145-8)²¹, anti- δ (TA4-1)²¹, anti- $\gamma 1$ (NL-16)²⁰, anti- $\gamma 2$ (GOM-2)²⁰, anti- $\gamma 3$ (C3-8-34)²², anti- $\gamma 4$ (9441-SA)²², anti- α (R1-29-2)²¹, anti- ϵ (RB6-2)³², anti- κ (TB28-1)²¹ and anti- λ (1-155-2)²¹ were used at concentrations previously determined to be optimal for indirect immunofluorescence^{21,22}. Slides were examined using fluorescence microscopy with epi-illumination and phase contrast optics. Pre-B cells were identified by their lymphoid morphology, lack of surface immunoglobulin and characteristic cytoplasmic immunofluorescence staining patterns^{10,21}. Results indicate the frequencies of positive cells among the total population of mononuclear cells with lymphoid morphology. More than 90% of the cells in the mononuclear cell preparation isolated by centrifugation on Ficoll-Hypaque density gradients exhibited lymphoblastic morphology. Minus sign (—) indicates <0.1%, and ND, not determined.

Table 2 Co-expression of immunoglobulin isotypes by γ^+ pre-B leukaemia cells

Patient	% γ^+ Pre-B cells that express:				
	μ	$\gamma 1$	$\gamma 4$	α	κ
C.Pr.	88	100	—	—	100
A.C.	98	100	4	—	80
C.Ph.	96	100	7	31	99
P.T.	97	100	—	—	100
M.K.	96	100	30	—	95
A.B.	100	100	21	—	98
C.R.	10	100	—	—	99
P.A.	2	96	37	—	100
P.K.	6	100	16	—	100

After fixation, blood or marrow mononuclear cells were doubly stained for cytoplasmic immunoglobulin components with mouse monoclonal antibodies specific for human heavy and light-chain isotypes, followed by rhodamine-labelled goat antibody to mouse immunoglobulins, and with fluorescein-labelled goat antibody to human γ chains; the latter antibody was extensively pre-absorbed with mouse immunoglobulins.

be located 5' to the $C_{\alpha 1}$ and $C_{\alpha 2}$ genes²⁹. Although a proposed gene order of μ , δ , $\gamma 1$, $\gamma 3$, $\gamma 2$, $\gamma 4$, ϵ and α would fit best with our observations, our data indicate that the switch sequence cannot be explained solely by the C_H gene order.

Isotype switching has not been detected in normal pre-B cells²¹. However, non-dividing immature B cells may express multiple heavy-chain isotypes^{10,11,30}, suggesting that these genetic decisions could be made even before the normal pre-B to B transition. In this case, the leukaemic pre-B-cell model may prove to be as informative as myeloma cells have been. Alternatively, chromosomal aberrations in the malignant pre-B cells could seriously complicate interpretation of our results. In this regard, karyotypic analysis of the leukaemic cells in all but two of the patients revealed normal chromosome numbers, except for hyperdiploid subpopulations of 57,XX and 58,XX chromosome containing cells in one patient and minor subpopulations of 45,XX and 90,XX cells in another. Specifically we did not note translocational abnormalities involving chromosomes 14 and 2, as have been observed in Burkitt's lymphomas expressing μ and κ genes³¹, but the present data do not address the possibility of smaller transpositional elements.

It was conceivable that in samples from individual patients we were observing multiple clones or subclones of the pre-B leukaemic cells each of which were undergoing different isotype switches. The leukaemic cells from three patients were examined using a panel of four monoclonal antibodies specific for V_H subgroups³², and in each case all of the identifiable pre-B cells reacted with the same monoclonal anti- V_H antibody. More compelling evidence for multiple isotype expression within a single leukaemic clone was obtained by two-colour immunofluorescence analysis of heavy-chain isotypes. The results indicate that individual pre-B cells expressed as many as three heavy-chain isotypes (Table 2), and possibly even four isotypes in one patient sample (C.Ph.). Thus multiple isotype switches were reflected within individual leukaemic clones. The stability of these phenotypic patterns has not been examined, but the presence of multiple heavy-chain isotypes in individual pre-B cells might be easily explained by the hypothesis of a preliminary switch mechanism involving a large primary transcript of all the C_H genes and differential RNA splicing^{19,23,33}. This hypothesis does not simplify the problem of ordered isotype switching in the leukaemic pre-B cells, but successful establishment of cell lines from these leukaemias could provide a useful model to examine the molecular basis of switching and its relation to the transformational event.

The simultaneous expression of κ light chains by almost all of the leukaemic pre-B cells exhibiting heavy-chain isotype switches is remarkable. This preference for κ over λ expression is perhaps not too surprising in view of previous evidence which suggests that V_{λ} - J_{λ} gene rearrangements for expression with C_{λ} regularly follow non-productive rearrangements of κ genes

on both chromosomes^{8,9}. However, the consistent acquisition of a productive κ gene by switching pre-B cells is unprecedented, and suggests that these genetic events occurring on separate chromosomes are coupled by a regulatory mechanism that remains to be elucidated.

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Use of iron- or selenium-coupled monoclonal antibodies to cell-surface antigens as a positive selection system for cells

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A system which confers selective growth advantage to cells expressing particular surface proteins would be extremely desirable, for such a technique would allow the study of receptors using somatic cell genetic techniques such as DNA-mediated cell transformation and selection of over-producing cell variants. Polypeptides bound to surface receptors, and antibodies bound to surface antigens generally are taken up efficiently by cells by endocytotic mechanisms. Several investigators have accordingly developed useful techniques for selection against cells expressing surface receptors and antigens, using hormones and antibodies conjugated to toxins^{1,2}. We reasoned that conjugation of nutrients to antibodies or hormones conversely might permit a positive selective pressure to be applied in appropriately constituted medium. We report here that monoclonal antibodies to cell-surface antigens will indeed deliver nutritional iron and selenium to cultured cells in an antigen-specific manner.

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We tested the feasibility of this approach using two monoclonal antibodies generated from mouse spleen-NS1 hybridomas. Hybridoma C10-7 secretes IgG1 which binds to an externally exposed plasma membrane protein antigen (PUNC) present on rat phaeochromocytoma PC12 cells but not on adult rat tissues nor on a PC12 variant, NR18A (ref. 3). Hybridoma 5A7 secretes IgM which binds to antigens found on PC12 cells and on NR18A and various rat tissues⁴.

Many cell lines can be maintained in chemically defined (serum-free) medium consisting of nutrient media plus various supplements, including insulin, transferrin and selenium which are required by most cell types⁵. Bottenstein and Sato⁶ reported that PC12 proliferate in medium supplemented with transferrin, insulin, selenium, putrescine and progesterone, although work in this laboratory (J. Strazdis, unpublished) demonstrates that PC12 cells grow equally well in the absence of putrescine and progesterone. We sought to determine whether cellular dependence on transferrin (presumably essential for iron uptake⁷) and SeO₂ could be by-passed by use of antibody derivatives containing iron or selenium. The use of such derivatives in medium deficient in transferrin or SeO₂ might be expected to select for cells expressing particular cell-surface antigens.

Antibodies used for these studies were prepared as ascites fluid from BALB/c mice inoculated intraperitoneally with hybridoma cells and were used without further purification. Iron was coupled to C10-7 IgG by conjugation with ferritin (see Fig. 2 legend for details). Ferritin was chosen because it functions physiologically for iron transport, yet most cells do not possess surface receptors for ferritin⁸. Indirect immunofluorescence microscopy (Fig. 1) shows that immunochemical specificity was retained by the ferritin-antibody conjugate (FeC10-7). FeC10-7 binds to PC12 cells but not to variant NR18A, which lacks PUNC antigen.

PC12 cells and NR18A cells grow well in RPM1 1640 supplemented with 50 $\mu\text{g ml}^{-1}$ transferrin, 5 $\mu\text{g ml}^{-1}$ insulin and 4 ng ml^{-1} SeO₂. We hypothesized that, with transferrin omitted, FeC10-7 would supply the nutritional iron requirement to PC12 cells and stimulate growth. To test this possibility, cells were cultured for 2 days in RPM1 1640 supplemented with only insulin and selenium to deplete internal iron pools and were then provided with medium additionally supplemented with transferrin, ferritin or FeC10-7. As shown in Fig. 2, PC12 cell growth increased with increasing FeC10-7 concentration and at 1.5 $\mu\text{g ml}^{-1}$ FeC10-7 it was comparable with that obtained with 100 $\mu\text{g ml}^{-1}$ transferrin, while unconjugated C10-7 antibody had no effect at comparable concentrations. Ferritin conjugated to non-immune mouse or rabbit IgG was also ineffective (data not shown). As seen in Fig. 2, unconjugated ferritin had a growth stimulatory effect but only at much higher concentrations than for the ferritin-C10-7 conjugate. Also shown in Fig. 2 are data indicating that the growth-stimulatory activity of FeC10-7 is removed by chromatography on protein-A-Sepharose, thus verifying that it is the antibody conjugate rather than some impurity which is effective. In a parallel experiment using NR18A cells lacking PUNC antigen, FeC10-7 had no growth-stimulatory effect. After 6 days of culture, NR18A cells numbers resulting from culture with no addition, and with 1 $\mu\text{g ml}^{-1}$ FeC10-7, were 30 and 33%, respectively, of the cell number obtained with 100 $\mu\text{g ml}^{-1}$ transferrin.

The selenium requirement may be exploited in a similar manner. Immunoglobulin containing selenium can be conveniently prepared by culture of hybridoma cells in medium containing selenomethionine rather than methionine. C10-7 hybridoma cells were grown in RPM1 1640 medium lacking methionine and supplemented with either 15 $\mu\text{g ml}^{-1}$ D,L-selenomethionine or 5 $\mu\text{Ci ml}^{-1}$ ⁷⁵Se-selenomethionine (Amersham) as well as with insulin, transferrin and selenium as above and 20 μM ethanolamine⁹. After 2 days, supernatant was collected and IgG was purified by protein A-Sepharose chromatography. IgG prepared in this manner contained selenium as shown in Fig. 3. Selenium-containing C10-7 IgG (SeC10-7) retained its binding specificity as shown by enzyme-

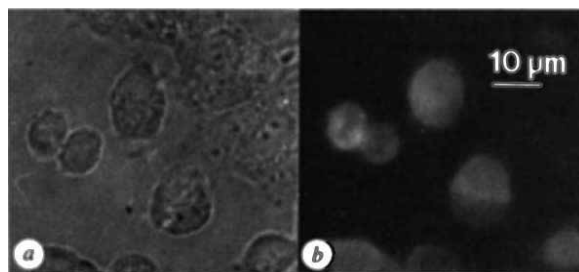


Fig. 1 Specificity of C10-7 antibody. PC12 cells and NR18A cells were allowed to attach to the same coverslip. Cells were fixed in 1% formaldehyde, incubated with 1% bovine serum albumin in phosphate-buffered saline and then treated with FeC10-7 followed by incubation with fluorescein-conjugated goat anti-mouse serum¹⁵. Cells were photographed using phase contrast (a) and epifluorescent (b) microscopy. Round PC12 cells can be distinguished from flat NR18A cells.

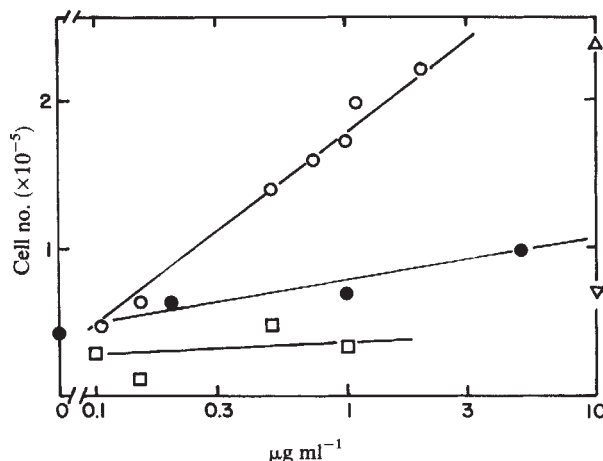


Fig. 2 Growth-promoting activity of FeC10-7. PC12 cells were seeded on rat tail collagen-coated 16-mm wells (2×10^4 cells per well) and were cultured for 2 days in RPM1 1640 supplemented with insulin (5 $\mu\text{g ml}^{-1}$) and selenium oxide (4 ng ml^{-1}). Medium was then additionally supplemented with various concentrations of ferritin (●), C10-7 antibody (□) or FeC10-7 (○). Cells were cultured for 6 more days after which cell number was determined using a Coulter counter. FeC10-7 conjugate was prepared by coupling C10-7 IgG to equine ferritin using glutaraldehyde¹⁶. Ferritin IgG conjugate (FeC10-7) was separated from unconjugated IgG by chromatography on Sepharose 4B. Unconjugated ferritin was separated from FeC10-7 by chromatography on *Staphylococcus aureus* protein A-Sepharose (Pharmacia). IgG-containing fractions were eluted with 0.1 M sodium citrate, pH 5.0. Purification was monitored by including ³⁵S-methionine-labelled C10-7 IgG as tracer. Symbols at the right extreme of the figure denote the cell number obtained with 100 $\mu\text{g ml}^{-1}$ transferrin (Δ) and with 0.5 $\mu\text{g ml}^{-1}$ of the protein which failed to absorb when the FeC10-7 preparation was chromatographed through protein A-Sepharose equilibrated with 0.1 M sodium phosphate, pH 8.0 (▽).

linked immunosorbent assays^{10,11} in which the antibody reacted with PC12 but not NR18A cells.

PC12 cells were cultured for 2 days in serum-free growth medium lacking selenium and SeC10-7 was then provided. Figure 3 shows that SeC10-7 effectively supported the growth of PC12 cells; however, SeC10-7 at these concentrations did not permit growth of NR18A (data not shown). Like SeO₂ (ref. 5), SeC10-7 is toxic above an optimum concentration, as shown by the decreased cell number at the highest SeC10-7 concentration in Fig. 3. This toxicity was more pronounced at SeC10-7 concentrations greater than plotted in Fig. 3.

The stringency of selection was increased by using antibody derivatives to supply iron and selenium simultaneously. PC12 cells showed no growth in RPM1 1640 supplemented only with

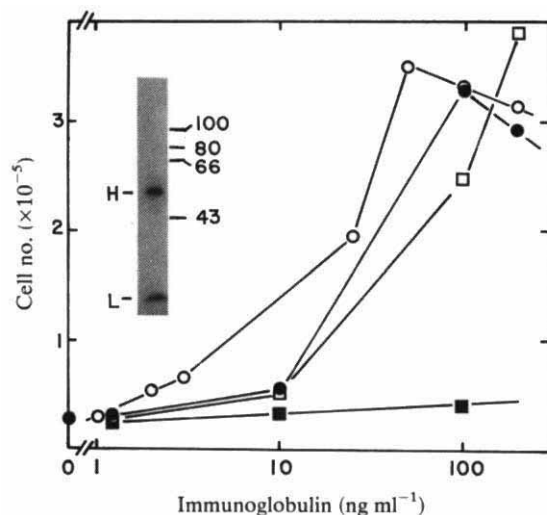


Fig. 3 Growth-promoting activity of SeC10-7 and Se5A7. Experimental design is as described in Fig. 2 legend except that PC12 cells were first incubated for 2 days in medium with insulin and transferrin and then for 6 more days with varying concentrations of the following additional supplements: C10-7 (unconjugated) (■), SeC10-7 (○), Se5A-7 (●); or cells were incubated for 2 days in medium supplemented only with insulin and then cultured for 6 more days with the addition of equal concentrations of SeC10-7 and FeC10-7 (□). Concentrations (ng ml^{-1}) are calculated in terms of immunoglobulin content of derivatives. The inset shows an autoradiogram of $^{75}\text{SeC10-7}$ after SDS-gel electrophoresis. Hybridoma cells were metabolically labelled with ^{75}S -selenomethionine as described in the text. Culture supernatant was collected, IgG was precipitated with formalin-fixed *S. aureus* cells (BRL) as described by Kennet¹¹ and absorbed protein was subjected to SDS-gel electrophoresis on 10% polyacrylamide gels¹⁷. H and L indicate heavy and light chains of IgG. The position of migration of marker proteins is indicated for ovalbumin (molecular weight 43,000), serum albumin (66,000), transferrin (80,000) and α -actinin (100,000).

insulin but proliferated rapidly in medium additionally supplemented with SeC10-7 and FeC10-7 (see Fig. 3). In fact, the extent of growth (15-fold in 8 days) obtained with 200 ng ml^{-1} (IgG content) SeC10-7 and FeC10-7 (Fig. 3) was identical to that obtained with medium containing optimum concentrations of SeO_2 and transferrin.

The ability of nutrient conjugates to stimulate cell growth is not limited to C10-7 antibody. Selenium-containing IgM from hybridoma 5A7 was prepared as described for C10-7, except that culture supernatant was prepared by dialysis against phosphate-buffered saline. The data in Fig. 3 show that Se5A7 efficiently replaces the requirement of PC12 cells for SeO_2 .

The results with these two antibodies demonstrate that antibody derivatives can effectively supply the nutritional needs of cells for iron and selenium, and do so in a manner which is specific for particular surface antigens. We propose the term 'nutrient portage selection' for this phenomenon, by analogy to 'portage transport' of substances into bacteria using their specific peptide transport systems¹². These techniques provide the basis for positive selection systems which should permit the isolation of DNA-mediated transformants expressing particular surface receptors. As a test of the efficiency of this selective system for isolating rare cells expressing a particular antigen from a population lacking the antigen, mixtures of PC12 and NR18A cells were subjected to culture in medium supplemented with insulin, selenium and FeC10-7. Three weeks of culture in this selective medium yielded homogeneous populations of PC12 cells from populations initially containing PC12:NR18A ratios as low as 1:10,000. (PC12 cells could be distinguished from NR18A cells on the basis of their morphology, see Fig. 1.)

Preliminary experiments have recently provided a more direct demonstration of the efficiency of the selection technique. We have subjected mouse Ltk⁻ cells to transfection with mixtures of PC12 DNA and plasmid DNA containing the herpes simplex virus thymidine kinase (*tk*) gene followed by selection in RPM1 1640 containing insulin and SeO_2 supplemented with FeC10-7 and hypoxanthine, aminopterin and thymidine (HAT). (The *tk* co-transfection with HAT selection exploits the anomalously high frequency of co-transformation by unlinked DNA sequences^{13,14}.) This protocol yielded clones simultaneously expressing HAT resistance and PUNC antigen at a frequency of 10^{-5} (T.B. and M.B., work in progress).

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Transcription initiation of Mu *mom* depends on methylation of the promoter region and a phage-coded transactivator

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The product of the bacteriophage Mu gene *mom* modifies adenine residues of DNA within the consensus sequence $\text{C}^{\text{A}}\text{G}^{\text{N}}\text{P}^{\text{y}}$, providing protection against various restriction $\text{G}^{\text{A}}\text{C}^{\text{C}}$ endonucleases (ref. 1 and D. Kamp, personal communication cited in ref. 2). The *mom* gene is only expressed during lytic development of the phage¹. It is known that *mom* is non-functional in *Escherichia coli* host mutants in a gene (*dam*) which itself encodes an adenine methylation system³⁻⁷. We show here that the *E. coli dam* gene is essential for transcription initiation of the *mom* gene, and that this dependence on *dam* seems to lie in a short segment preceding the *mom* coding region, which also contains the *mom* promoter. The sequence of this segment reveals the presence of *dam* methylation sites (GATC), and suggests a model for the regulation of *mom* gene expression based on DNA secondary structure, which may explain why *mom* is only expressed during phage lytic development. We also show that expression of phage-coded proteins (A, B and C) is needed for transactivation of *mom* transcription.

We have cloned the invertible G region of Mu and the adjacent β -region into pBR322 (ref. 8). From this plasmid we constructed a subclone, pGP203, which was used to make a restriction map of the β -region (Fig. 1). As the DNA between the invertible region and the *PvuI* site is known to code for the

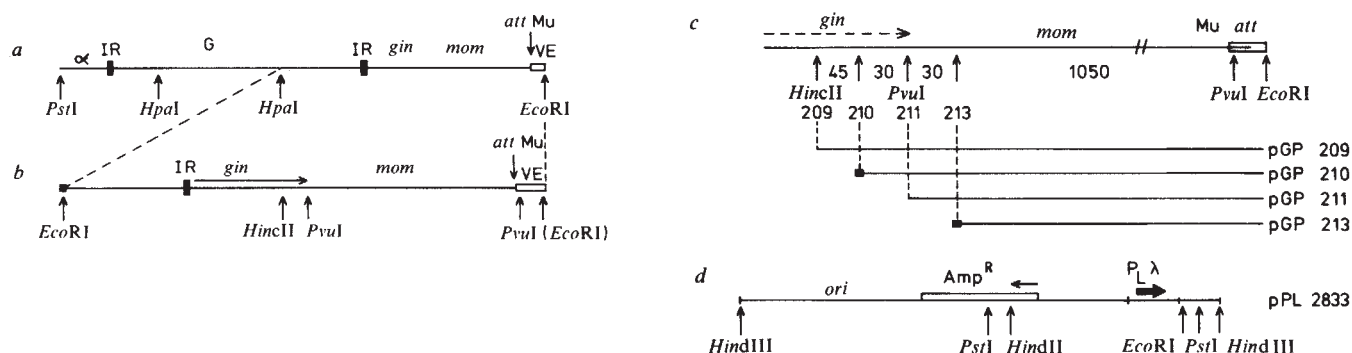


Fig. 1 Linear map of the Mu invertible region and β -region cloned on pBR322. Subclones of the Mu β -region containing *mom*. *a*, Linear representation of the Mu DNA on pGP56⁸. The inverted repeats (IR) flanking the G region are indicated by bars. In the adjacent β -region two non-essential genes have been mapped: *gin*¹⁹ and *mom*⁵. VE indicates the variable end of Mu. *b*, Linear representation of pGP203: the *HpaI*-*EcoRI* fragment of pGP56 was cloned into the *EcoRI* site of pBR322, after ligation of an *EcoRI* linker to the *HpaI*-generated blunt end. The orientation is such that the Mu attachment site is closest to the *ter'* gene. One *EcoRI* site was removed by filling up the sticky ends using Klenow fragment of DNA polymerase (indicated by brackets). *c*, Part of the β -region (as cloned in pGP203) is shown in the top line. The Mu DNA contains at its end a small piece of host DNA, the variable end, indicated by an open bar. The distances between the sites are indicated (determined by agarose gel electrophoresis). Subclones of pGP203 were made as follows: pGP203 was partially cut with *HincII*, and the appropriate fragment purified from an agarose gel²⁰. After ligation, *ter' amp'* transformants were selected. In the resulting plasmid, pGP209, the *HincII* site in *gin* is ligated to the *HincII* site in the β -lactamase gene of pBR322 (coordinate 3,908; ref. 21). A similar procedure was followed using *PvuI* instead of *HincII*; in the resulting plasmid, pGP211, the *PvuI* site 211 is ligated to the *PvuI* site in the β -lactamase gene of pBR322 (coordinate 3,737; ref. 21). By using *Bal31* nuclease (Boehringer, Mannheim) deletions were made from the *EcoRI* site in pGP203, and the *EcoRI* linker GGAATTCC was ligated to the deleted molecule (indicated by solid bars). The deletions extended into the β -lactamase gene approximately to position 3,600 (ref. 21) and into the β -region as indicated. The *EcoRI* linkers are represented by a solid bar. The exact position of sites 210 and 211 was determined by DNA sequencing (see Fig. 2). *d*, The *mom* gene on the plasmids pGP209 and pGP213 was brought under the expression of the λ *p_L* promoter. The plasmid pPL2833 (ref. 15) carries an *amp'* gene as in pBR322 and the *p_L* promoter (plus operator) of phage λ , 200 bp downstream of the promoter, is a linker containing several restriction sites. This promoter was inserted into sites 209 and 211 by inserting the *PstI* fragment of pPL2833 into the *PstI* site of pGP209 and pGP211 so that the *amp'* gene was restored. Note that a small piece of pBR322 DNA is left between the *p_L* promoter and sites 209 and 211 (300 and 120 bp, respectively). In the case of pGP210 and pGP213 the *EcoRI*-*HindIII* fragments of these plasmids were inserted into the sites in the linker of pPL2833. After transformation to the strain NFI (containing a λ prophage deleted for all functions except *cI857*; ref. 15) *amp'* colonies were selected at 28°C. Strains were grown at 28°C to keep the *p_L* promoter under repression of the thermosensitive *cI* repressor.

gin gene of Mu⁹⁻¹¹ we further subcloned the 1,275-base pair (bp) fragment between the *HincII* site in *gin* and the Mu attachment site and the 1,200-bp fragment between the *PvuI* site and the attachment site (pGP209 and pGP211; Fig. 1a, b). These clones were tested for Mom expression: an *E. coli* K-12 strain containing one of these plasmids was infected with a Mu *mom* phage, and after phage development the lysate was titrated on an *E. coli* K-12 strain containing additional restriction systems. Table 1 shows that the titre of a Mu *mom* on such a strain is 10⁴ less than that of a wild-type Mu. Mu *mom* grown on a strain containing pGP203, pGP209 or pGP211 shows the same efficiency of plating (EOP) on a restricting strain as a wild-type Mu. Thus, the *mom* mutation is complemented by these plasmids, and *mom* maps to the right of the *PvuI* site.

Using the exonuclease *Bal31*, various deletion derivatives were made from the plasmid pGP203. Two of these deletions are shown in Fig. 1c: they extend, respectively, to the indicated sites 210 and 213. The clone pGP210 is Mom⁺, whereas clone pGP213 is Mom⁻ (see Table 1). Thus, at least part of the signal sequences of *mom* are mapped between the *PvuI* site (pGP211) and site 213.

It has been suggested that another Mu function may be essential for Mom expression⁵. The ability of the described plasmid clones to express *mom* in the absence of a Mu helper phage was determined by carrying out an experiment similar to that described above, using phage λ instead of Mu *mom*. In this case no Mom protection against modification could be detected (Table 1). This cannot be due to an inability of Mom to modify λ DNA, as it has been shown that in a mixed infection with both phages Mu and λ , Mom also modifies the λ DNA¹. Therefore, this experiment shows that a second Mu function is essential for Mom; this could be either a transactivator of the *mom* gene or a second factor involved in the modification

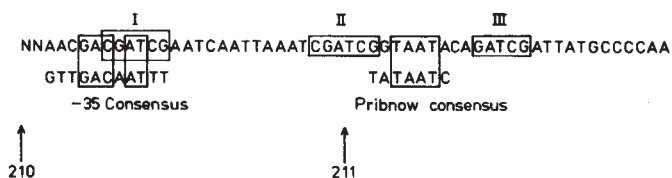


Fig. 2 Sequence of the DNA segment determining Dam dependence. The sequence of the region determining Dam dependence of Mom expression and the end points of deletions 210 and 211 were determined using the M13 cloning and dideoxy sequencing system. The *EcoRI*-*HindIII* fragment of pGP210 was cloned into the RF form of phage M13mp9 (ref. 22). The polymerization reaction was done as described elsewhere²³, and the DNA was separated on 12.5% polyacrylamide gels²⁴. The three regions of palindromic repeats are indicated by boxes I, II and III. There are *PvuI* sites in boxes I and II. The sequence to the right of site 210 was screened by hand for sequences matching the consensus promoter sequence. The best fit is indicated; sequences common with the consensus are boxed. Sites 210 and 211 are indicated. The two bases immediately following the *EcoRI* linker in pGP210 have not been determined.

process itself. To characterize this function we tested a set of Mu mutants, each having an amber mutation in one essential gene^{12,13}, for their ability to express Mom. Table 2 shows that all Mu amber mutants tested express *mom*, except for *Aam*, *Bam* and *Cam*. Genes *A* and *B* are essential for replication of the phage; after the onset of replication *C* is expressed, which is essential for turning on all the late functions of Mu¹⁴. As replication in a *Cam* mutant is normal it may be concluded that replication of the phage alone is insufficient for Mom

Table 1 Expression of Mom in plasmid clones

Strain	Plasmid	Mu mom			λ		
		Titre on r^-	Titre on r^+	EOP	Titre on r^-	Titre on r^+	EOP
HB101	-	2.0×10^9	2.0×10^3	1×10^{-6}	1.0×10^{10}	3.4×10^4	3×10^{-6}
HB101	pGP203	3.0×10^9	2.0×10^8	2×10^{-1}	1.5×10^{10}	3.4×10^4	2×10^{-6}
HB101	pGP209	3.0×10^9	1.6×10^8	5×10^{-2}	1.0×10^{10}	3.2×10^4	3×10^{-6}
HB101	pGP210	4.0×10^8	8.1×10^7	2×10^{-1}	1.2×10^{10}	3.2×10^4	3×10^{-6}
HB101	pGP211	2.5×10^9	1.2×10^8	5×10^{-2}	1.5×10^{10}	3.4×10^4	2×10^{-6}
HB101	pGP213	3.2×10^8	5.0×10^3	1×10^{-5}	1.5×10^{10}	3.0×10^4	2×10^{-6}
Mom under expression of a foreign promoter							
NF1	pPL3833	1.8×10^9	2.0×10^3	1×10^{-6}	1.5×10^{10}	6.7×10^4	5×10^{-6}
NF1	pGP209	1.7×10^9	1.3×10^8	8×10^{-2}	1.5×10^{10}	1.0×10^5	7×10^{-6}
NF1	pGP211	2.0×10^9	1.6×10^8	8×10^{-2}	1.5×10^{10}	1.0×10^5	7×10^{-6}
NF1	pGP213	1.2×10^{10}	1.4×10^4	1×10^{-6}	1.0×10^{10}	5.0×10^4	5×10^{-6}
NF1	pGPPL203	4.2×10^9	1.6×10^8	4×10^{-2}	1.3×10^{10}	1.5×10^7	1×10^{-3}
NF1	pGPPL209	2.0×10^9	2.5×10^6	1×10^{-3}	5.0×10^9	2.5×10^9	5×10^{-1}
NF1	pGPPL210	4.5×10^8	6.0×10^5	1×10^{-3}	3.0×10^9	2.6×10^8	9×10^{-2}
NF1	pGPPL211	1.0×10^9	1.3×10^6	2×10^{-3}	5.2×10^9	2.5×10^9	5×10^{-1}
NF1	pGPPL213	1.9×10^9	1.5×10^6	8×10^{-4}	2.5×10^9	1.8×10^8	7×10^{-2}
Expression of Mom of plasmid clones in a Dam ⁻ background							
KA816(dam)	-	1.6×10^{10}	3.0×10^2	2×10^{-8}	1.1×10^{10}	3.0×10^4	3×10^{-6}
KA816	pGP209	5.6×10^9	2.2×10^3	4×10^{-7}	1.2×10^{10}	3.0×10^4	3×10^{-6}
KA816	pGP210	1.2×10^{10}	6.4×10^6	5×10^{-4}	8.5×10^9	2.0×10^4	2×10^{-6}
KA816	pGP211	1.0×10^{10}	4.0×10^8	4×10^{-2}	1.6×10^{10}	5.0×10^4	3×10^{-6}
KA816	pGP213	3.7×10^9	1.3×10^3	4×10^{-7}	1.2×10^{10}	1.0×10^5	8×10^{-6}
KA816	pGPPL209	2.6×10^9	1.0×10^9	4×10^{-1}	1.1×10^{10}	1.3×10^8	1×10^{-2}
KA816	pGPPL210	2.0×10^7	3.0×10^7	1	6.0×10^9	3.0×10^9	5×10^{-1}
KA816	pGPPL211	3.3×10^9	3.0×10^8	9×10^{-2}	1.1×10^{10}	1.6×10^8	2×10^{-2}
KA816	pGPPL213	3.0×10^7	5.0×10^5	2×10^{-2}	6.0×10^9	1.8×10^9	3×10^{-1}

E. coli K-12 strains HB101 containing the different plasmids were grown to A_{600} of 0.2 and infected with Mucts62, mom3452 or λ at a multiplicity of infection (MOI) of 5. After 90 min at 37°C the cell debris was spun down and the titres of the phages in the supernatant determined on the r^- strain HB101 (*hsD*), and the r^+ strain 2379 (*P1*) containing, respectively, the restriction modification systems *hsA* and *hsP1*. Phage and bacterial growth conditions were as described elsewhere¹⁸. The EOP of a wild-type Mu (grown by induction of a lysogen) on a r^+ strain is 5×10^{-2} . The strain NF1 contains a λ prophage deleted for all functions except the thermosensitive repressor gene *cI857*. The strains containing the pGPPL plasmids were grown at 28°C to A_{600} 0.2 and induced at 42°C for 15 min before infection with Mu mom and λ . Phage were allowed to develop at 37°C for 90 min. Experiments with pGPPL plasmids in KA816 (*dam3*) were done using the compatible plasmid pCI857. The plasmid pCI857 is a pACYC177 derivative carrying the *cI857* gene of phage λ ¹².

Table 2 Mom expression of Mu phage mutants

Strain	Relevant genetic markers	Titre on r^-	Titre on r^+	EOP on r^+
KA52	-	3×10^9	2×10^5	7×10^{-5}
KA52 + pGP203	-	4×10^9	1×10^5	5×10^{-5}
KMBL2302	::Mucts62, <i>Aam</i> 1093	3×10^9	5×10^4	2×10^{-5}
KMBL2304	::Mucts62, <i>Bam</i> 1066	5×10^9	5×10^4	1×10^{-5}
KMBL2332	::Mucts62, <i>Cam</i> 2005	5×10^9	5×10^4	10^{-5}
KMBL2330	::Mucts62, <i>Sam</i> 1004	1×10^9	1×10^7	10^{-2}

The different Mucts lysogens were grown at 32°C and at A_{600} of 0.2 they were shifted to 42°C, 15 min after the temperature shift, the cells were infected at a MOI of 5 with λ phage. After 90 min growth at 37°C, the lysates were titrated on bacterial strains HB101 and 2379 (*P1*). Similar results as shown here for Mucts62, *Sam* were obtained for the Mu phages tested having an amber mutation in the genes *lys*, *E*, *G*, *H*, *I*, *J*, *K*, *L*, *M*, *N*, *O*, *P*, *Q*, *R*, *T*, *V* and *W*.

expression. Probably the *mom* gene is turned on directly or indirectly by *C*.

To test whether the second Mu function is essential in the actual modification reaction or for turning on the *mom* gene, the latter was fused to another promoter. The P_L promoter of bacteriophage λ was inserted into different plasmids carrying *mom* (Fig. 1d), by ligating a DNA fragment carrying this promoter¹⁵ into various restriction sites localized before *mom*. These plasmids were tested for their ability to express *mom* (after thermoinduction of the repressor *cI857*) in the presence and absence of a Mu helper phage; Table 1 shows that the plasmids express *mom* in the absence of Mu growth, that is, without transactivation. This demonstrates that: (1) the second Mu function involved in Mom modification is a transactivator,

positively controlling transcription of the *mom* gene. (2) When *mom* is transcribed from a foreign promoter, the Mu transactivator is no longer needed for *mom* transcription. Therefore the transactivator is probably involved in transcription initiation rather than in antitermination. (3) The promoter of *mom* is located between the *PvuI* site and site 213, and the translation start is to the right of site 213, as insertion of a foreign promoter into this site fully restores the *mom* complementation. (For unknown reasons the EOP of Mu *mom* grown on pGPPL strains is rather lower than when it is grown on pGP strains.)

Using the plasmids pGP209 and pGPPL209 we analysed another aspect of the regulation of Mom expression. These plasmids show a marked difference in their behaviour in a Dam⁻ strain. The adenine methylating Dam function was previously shown to be essential for Mom expression, and it was assumed that it would cooperate in the modification reaction⁷. Recently it has been shown, however, that the Dam methylase has a role in the transcription of the *mom* gene¹⁶. Indeed, Table 1 shows that pGP209 does not complement a Mu *mom* in a Dam⁻ background. However, pGPPL209 normally expresses Mom in a Dam⁻ background, as does pGPPL213. This shows that Dam is essential for transcription of the *mom* gene, but it is not essential in the modification reaction. The only conceivable way that Dam could affect *mom* transcription seems to be methylating signal sequences of *mom* in order to allow *mom* transcription. The positive effect of Dam on *mom* transcription must be on transcription initiation because insertion of a promoter to the left of *mom* results in Dam independence. When tested in a Dam⁻ strain, the plasmid pGP211 expressed *mom* (has its own promoter and still needs transactivation). It can be concluded that the ~75 bp between the *HincII* site and the *PvuI* site contain a sequence which prevents *mom* transcription in a Dam⁻ strain. Probably methylation of this sequence is needed for *mom* transcription. Deletion of this sequence abolishes the Dam dependence. Note that the C-terminal end

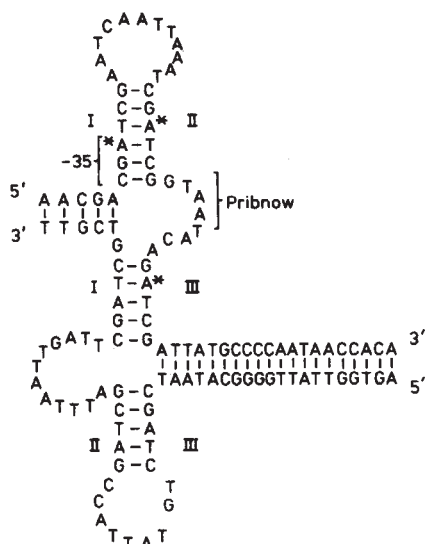


Fig. 3 Hypothetical secondary of the Mu *mom* promoter region. This is one of the three possible structures in which all six boxes are in a duplex. The numbers of the boxes are as in Fig. 2. The putative -35 region and Pribnow box are indicated. Methyl groups are indicated in one of the two strands by an asterisk. In this figure only the coding strand is methylated.

of the preceding *gin* gene is also in this 75-bp segment^{9,10}. The sequences before the *PvuI* site were deleted for pGP210, leaving only the last 30 bp; its *Mom* expression was still Dam-dependent (though slightly reduced). This maps the Dam dependence to the 30 bp immediately preceding the *PvuI* site.

The DNA segment containing the Dam-dependence site and the *mom* promoter was sequenced (see Fig. 2). Some features are immediately clear—instead of one there are two *PvuI* sites close to each other. This region contains three palindromic repeats (indicated by boxes) and within each repeat is a GATC sequence, which is the target site of Dam methylation. The presence of these GATC sites close to the end of the *gin* gene was first reported by Kahmann¹⁰. In this region a rather good promoter sequence can be found matching four of the seven base pairs with the consensus Pribnow box, and five with the -35 region. The putative Pribnow box is just to the right of the second *PvuI* site. This is in agreement with the fact that in the clone pGP211 (*Mom*⁺, Dam-independent) the Pribnow box has not been deleted, but the Dam-dependence region has. (By sequencing we confirmed that in pGP211 the DNA up to the second *PvuI* site had indeed been removed.) In this multicopy plasmid, deletion of the -35 region apparently does not abolish promoter activity. It is not surprising that the putative promoter is far from perfect compared with the consensus sequence, as it is known that it only promotes transcription initiation in the presence of a positive regulator (the Mu transactivator; see also ref. 5).

The following picture of the regulation of *Mom* expression emerges from these experiments. *Mom* has its own promoter, located between the second *PvuI* site and site 212, that is, a promoter to the right of the *gin* gene, but quite close to it. It is probably localized between boxes II and III of the region with triad symmetry. The target site for the transactivation is also mapped to the right of the *PvuI* sites. It has not been possible to separate the *mom* promoter and the site determining transactivation dependence. Together with the finding that *mom* transcription from a foreign promoter is transactivation-independent, this strongly suggests that the transactivator is directly involved in transcription initiation. The site determining Dam dependence is mapped overlapping the putative -35 region and the Pribnow box. In pGP211 one of the three repeats

has been removed, but the Pribnow box is left intact, leading to Dam-independent *Mom* expression.

The sequence of the promoter region suggests a structural basis for the Dam dependence. Two alternative structures in double-stranded (ds)DNA are envisaged in which the three boxes on both strands are all in a duplex (one of these is shown in Fig. 3). The stability of this hypothetical structure may depend on the presence of methyl groups in (some of) the six boxes. If such a structure is involved in the process of transcription initiation this may account for the dependence of Dam methylation. This raises the question of the function of the Dam dependence. As stated above, *Mom* is only expressed during lytic development of the phage, and the replication genes *A* and *B* are essential for *Mom* expression. It is known that the newly synthesized strand is not methylated immediately¹⁷. Possibly the secondary structure triggering transcription initiation can only be adopted by hemimethylated DNA, that is, DNA of a replicating phage, implying that both fully and non-methylated promoters are not expressed. This would be an accurate means of regulating *mom* expression, and would reflect the situation in mammalian cells where hypomethylation is believed to precede active transcription.

The function of both transactivation of *Mom* expression and this hypothetical replication-dependent transcription initiation can be understood by the fact that the constitutive expression of *Mom* in a lysogen probably would be lethal for the host. *Mom* modifies about 15% of all A residues in the DNA⁶. We have found that only partial induction of *mom* (cloned on the plasmid pGPPL211) is already lethal to the host (results not shown).

Different phenotypic effects of the Dam methylase have been reported⁴, and in view of the results described here, some effects may possibly be attributed to the indirect effect of Dam on the expression of other *E. coli* genes.

During this work on Dam dependence we were informed that Dr R. Kahmann had made *mom* independent of Dam by deleting a region upstream of the gene.

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Sequences homologous to *Agrobacterium rhizogenes* T-DNA in the genomes of uninfected plants

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Agrobacterium rhizogenes causes the plant disease known as hairy root. Like crown gall disease, which is caused by *Agrobacterium tumefaciens*, hairy root disease is characterized by the proliferation of plant tissue at a wound site on infection by the bacterium. In both diseases, a large plasmid in the bacterium encodes essential virulence traits¹⁻⁴, and a portion of the plasmid is transferred, maintained and expressed in the plant genome⁵⁻¹⁵. The transferred DNA, or T-DNA, encodes the synthesis of opines and, in an unknown manner, confers phytohormone-independent growth¹⁶⁻²¹. Virulence in *A. rhizogenes* strain A4 is associated with the 240 kilobase (kb) Ri (root-inducing) plasmid pRiA4b (ref. 4). The DNA from callus and root culture derived from infections by this strain contain T-DNA from a 20 kb region of the plasmid¹⁴. A surprising feature of pRiA4b is the homology between plasmid sequences and the genome of the untransformed host plant *Nicotiana glauca*¹⁴. Previous evidence suggested that the homologous plasmid-plant sequences were in or near the T-DNA region of the plasmid¹⁴. We now present evidence that the homologous sequences are indeed within the T-DNA of the Ri plasmid, and that at least one genetic locus on the corresponding plasmid sequences functions in the induction of the hairy root disease.

The cosmid pNW44 contains ~40 kb of DNA from pRiA4b (see Fig. 1). This clone was shown previously to include both the T-DNA region and the sequences that hybridize to the uninfected plant genome¹⁴. The known T-DNA region extends from within a 6.2 kb *Hind*III fragment (H-11) to a 5.0 kb *Hind*III fragment (H-16) (F. W. *et al.*, unpublished data). An additional 18 kb of sequences adjacent to H-11 and H-16 are also present on pNW44. The location of the plant-homologous sequences within this segment of the Ri plasmid was first determined by probing plant DNA with individual *Hind*III fragments subcloned from pNW44. These subclones were hybridized to Southern blots of DNA from both transformed and uninfected plant tissues, digested with either *Hind*III or *Kpn*I. Reconstruction experiments were performed by the addition of pRiA4b DNA to uninfected plant DNA before digestion with *Hind*III. Figure 1 presents the results for subclones of fragments H-17 (probe A) and H-16 (probe B). When the 4.3 kb H-17 was used as the probe in the analysis of DNA from transformed *N. glauca*, the fragment was detected in multiple copies in *Hind*III digested DNA (Fig. 1a, lane 2). Similarly, H-17 spans the junction of two *Kpn*I fragments, K-8 (11.2 kb) and K-12 (5.8 kb), both of which were detected as internal fragments of the T-DNA (Fig. 1a, lane 3). Numerous other fragments were also detected in both the *Hind*III and *Kpn*I digests and these fragments may represent copies of this region that are covalently joined to plant DNA sequences. These results are consistent with previous data suggesting that H-17 of pRiA4b is in the core region of the T-DNA¹⁴.

When DNA from uninfected plant tissue was annealed with H-17, hybridization to all of the normal plant DNA fragments that share homology with pRiA4b and pNW44 was observed. An 8.2 kb *Hind*III fragment, found previously in normal plant DNA, was observed in addition to the expected 4.3 kb plasmid fragment (Fig. 1a, lane 1) in the reconstruction experiment. All three of the *Kpn*I fragments of normal plant DNA (12.5, 10.0

and 6.0 kb, respectively) previously observed to hybridize with pRiA4b hybridized with H-17 (Fig. 1a, lane 4). In similar experiments, fragments H-30a and H-32, which are contiguous with H-17, also shared homology with the plant DNA (data not shown). Other *Hind*III fragments from pRiA4b were hybridized with plant sequences. For example, the fragment of the T-DNA, H-16 (probe B, Fig. 1), did not hybridize to any normal plant DNA fragments. H-16 did hybridize to the 5.0 kb plasmid fragment in *Hind*III digested DNA of the reconstruction experiment (Fig. 1b, lane 1) and the numerous fragments that contain portions of H-16 present in transformed tissue (Fig. 1b, lanes 2 and 3). Homology to normal plant sequences was not observed for fragments extending 40 kb to either side of *Hind*III 17, 30a and 32 (data not shown). Fragments H-17, H-30a and H-32 account for all the fragments observed in hybridization to normal plant DNA digested with *Kpn*I. These results clearly showed that the plant-homologous sequences were situated within the T-DNA region of the Ri plasmid. We

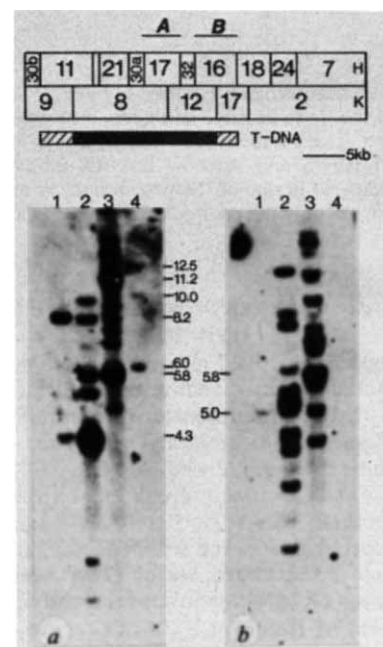


Fig. 1 Homologous sequences in the T-DNA region of the Ri plasmid. A map of the *Hind*III fragments (H) and the *Kpn*I fragments (K) of the region of pRiA4b present in pNW44 is shown in the upper portion of the figure. The vector fragment (6.4 kb) is not included. The probes, designated A and B, consisted of *Hind*III fragments 17 and 16 cloned into pHC79²⁴ and were used for the Southern analysis²⁵ shown in panels a and b, respectively. Lane 1, DNA from uninfected *N. glauca*, with pRiA4b DNA added equivalent to approximately one copy of the plasmid per diploid *N. glauca* genome¹⁴, digested with *Hind*III; lane 2, DNA from a root culture initiated by infection with *A. rhizogenes* strain A4 digested with *Hind*III; lane 3, transformed root DNA digested with *Kpn*I; lane 4, uninfected *N. glauca* DNA digested with *Kpn*I. Each lane represents a 10 µg sample of DNA digested in a 50 µl reaction volume with the appropriate enzyme (*Hind*III or *Kpn*I). The numbers between panels a and b indicate the fragment size in kilobases (kb).

Methods: Gel electrophoresis and the conditions of hybridization were essentially those described previously^{6,14}. After digestion, the DNA was loaded into wells in a submerged 0.7% agarose gel (0.7 cm thick, 38 cm long), subjected to electrophoresis at 1.0–1.3 V cm⁻¹ for 30 h, transferred to nitrocellulose filters²⁵ and hybridized with the appropriate probe. The probes were labelled with [α -³²P]dNTP to high specific activity (1.0–5 × 10⁶ c.p.m. per µg) by nick translation²⁶. Hybridization reactions were in 3 × SSC at 68 °C for 36 h and the filters were washed in 0.3 × SSC at 68 °C as described by Thomashow *et al.*⁶. The filters were air-dried and exposed on Kodak RP X-Omat film with intensifying screens at –80 °C.

hereafter refer to the sequences in the plant as the cellular T-DNA (cT-DNA).

To characterize the cT-DNA in greater detail, a region of the plant genome containing cT-DNA sequences was cloned into the vector λ 1059. The clone λ Ng31, which contained ~22 kb of plant DNA, was detected in a *Sau*3a partial library of the *N. glauca* genome using a 32 P-labelled pRiA4b probe. When digested with *Kpn*I, DNA from λ Ng31 yielded six fragments, two of which were phage arms ligated to plant DNA (Fig. 2a, lane 1). Three of the *Kpn*I fragments shared homology with pRiA4b (Fig. 2a, lane 2). These were the 10.0 and 6.0 kb plant DNA fragment, which probably represented the fragments of the same size as detected in the genomic Southern analysis (Fig. 1, lane 4), and a 13.5 kb *Kpn*I fragment that represented a phage arm-plant DNA junction. This fragment may have contained only a portion of the 12.5 kb *Kpn*I fragment observed in genomic DNA. (Size limitations of the λ 1059 vector did not permit inclusion of all three *Kpn*I fragments within one clone.) When λ Ng31 DNA was radioactively labelled and used as a probe of pNW44 or pRiA4b, only three *Hind*III fragments were detected (Fig. 2b, lanes 1 and 2, respectively). These were the same fragments, namely H-30a, H-17 and H-32, that hybridized to DNA from uninfected plants. No additional fragments of pRiA4b were detected. To determine whether the

entire plant complement of homologous DNA was obtained, λ Ng31 DNA was hybridized to total *N. glauca* DNA. In addition, λ Ng31 DNA was hybridized to DNA from transformed tissue in order to detect possible rearrangements of the cT-DNA region in hairy root tissue. These experiments, however, were inconclusive. The low copy sequences of the cT-DNA (1–2 copies per haploid genome) could not be detected due to the presence of one or more copies of highly repetitive plant DNA sequences in λ Ng31, which caused overexposure of the autoradiogram (Fig. 3c). Further characterization of the cT-DNA will require subcloning of the plant DNA of λ Ng31 to separate the unique sequence DNA from highly repetitive DNA.

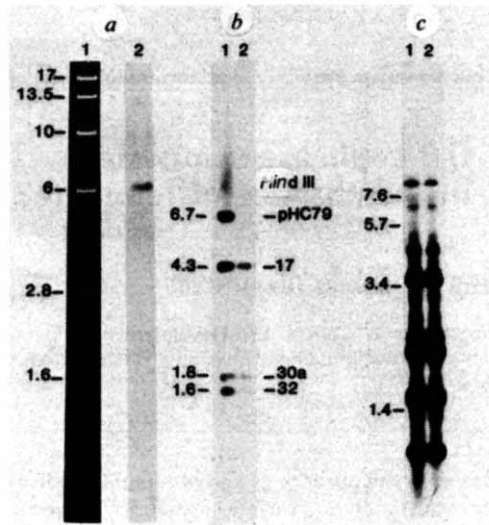


Fig. 2 Characterization of recombinant phage λ Ng31, which contains sequences homologous with pRiA4b T-DNA. **a**, A photograph of an ethidium bromide-stained 0.7% agarose gel which contains *Kpn*I-cleaved λ Ng31 DNA (lane 1), and an autoradiograph of the resulting nitrocellulose filter after Southern transfer and hybridization with 32 P-labelled pRiA4b (1.2×10^8 c.p.m. μg^{-1}) (lane 2). **b**, Hybridization of 32 P-labelled λ Ng31 DNA (2.5×10^8 c.p.m. μg^{-1}) with filter-bound *Hind*III-cleaved pNW44 (lane 1) and pRiA4b (lane 2). **c**, Hybridization of 32 P-labelled λ Ng31 DNA (2.5×10^8 c.p.m. μg^{-1}) with filter-bound *Hind*III-cleaved DNA from uninfected *N. glauca* leaves (lane 1) and transformed *N. glauca* roots (lane 2). The numbers to the left of the figures refer to fragment size in kb. The map locations of *Hind*III fragments 17, 30a and 32 are shown in Fig. 1.

Methods: The recombinant phage was constructed by ligating *Sau*3a-cleaved *N. glauca* leaf DNA (obtained by partial digestion and purification of molecules ~18 kb long using preparative agarose electrophoresis²⁷) to *Bam*HI-cleaved λ 1059 as described by Karn *et al.*²⁸ (λ 1059 DNA was a gift from E. Selsing). After *in vitro* packaging of the recombinant phage pool²⁹ and plating on the restrictive host strain Q364, 1 plaque out of 400,000 hybridized with 32 P-labelled pRiA4b. Clonally purified recombinant phage were grown as liquid lysates on *Escherichia coli* strain CSH18 and phage DNA was isolated as described previously²⁹. Additional nucleic acid manipulations were performed as described for Fig. 1.

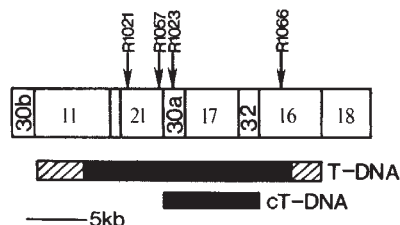


Fig. 3 Location of Tn5 insertions into the T-DNA region. Tn5 insertions into the Ri plasmid were obtained by infecting *E. coli* strain HB101, containing the T-DNA region cloned into the wide host range cosmid pHK17³⁰, with a defective $\lambda :: \text{Tn}5$ ³¹. Cosmids containing Tn5 were selected by *in vivo* packaging, transduction to a new host, and plating the transductants on L agar containing 50 $\mu\text{g ml}^{-1}$ kanamycin (Sigma) and 10 $\mu\text{g ml}^{-1}$ tetracycline (Sigma). Resistance to kanamycin and tetracycline was conferred by Tn5 and pHK17, respectively. The positions of Tn5 in the cosmids were analysed according to the quick screening method of Birnboim and Doly³². The mutations then were moved onto the Ri plasmid by the method of marker exchange³¹ as modified by Garfinkel *et al.*²⁰

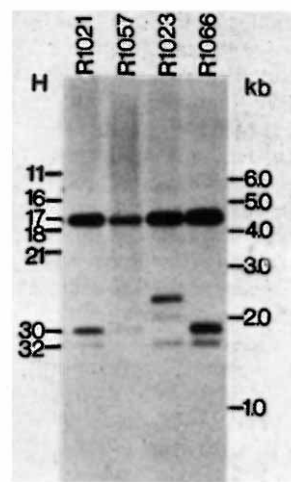


Fig. 4 Location of Tn5 insertion affecting virulence within the cT-DNA region of the Ri plasmid. The *Hind*III (H) fragments are designated on the left side while a calibration of fragment size in kb is indicated on the right side of the figure.

Methods: Total DNA from *A. rhizogenes* strains R1021, R1057, R1023 and R1066 was isolated by the procedure described by Currier and Nester³³. The DNA was digested with *Hind*III and subjected to electrophoresis on a horizontal 0.7% agarose gel (0.5 cm thick, 22 cm long) for 15 h at 30 V. Electrophoresis buffer was Tris-borate (0.089 M Tris, pH 8.0, 2.5 mM Na_2EDTA , 8.9 mM boric acid). The DNA was transferred to nitrocellulose and hybridized with a [α - 32 P]-labelled λ Ng31 as described in Fig. 1.

Several conclusions can be drawn from these experiments. The cT-DNA region was contained entirely within the contiguous *Hind*III fragments H-30a, H-17 and H-32 of the Ri plasmid T-DNA. It is interesting that all of these fragments are present in multiple copies in transformed plant tissue. At least a significant portion of the cT-DNA resided in one region of unique or low copy plant sequences in the *N. glauca* genome. Preliminary mapping data suggest that the 12.5, 10.0 and 6.0 kb *Kpn*I fragments of the plant are contiguous, and are represented in λ Ng31 by the 10.0 and 6.0 kb *Kpn*I plant DNA fragments and a portion of the 13.5 kb *Kpn*I phage-plant DNA fragment. Associated with the unique sequence DNA were sequences that were members of highly repeated DNA families.

It will be interesting to determine the biological function of the cT-DNA. Evidence that the cT-DNA region of the Ri plasmid encodes a gene concerned with hairy root induction comes from a mutational analysis of the genes responsible for virulence. Several Tn5 insertions in the Ri plasmid were obtained that mapped within the T-DNA region (Fig. 3). One insertion mutant, R1023, contained Tn5 inserted into fragment H-30a. Whereas the wild-type strain R1000 incited typical hairy root symptoms on the leaves of *Kalanchoe daigremontiana*, the insertion in H-30a resulted in the loss of virulence. Since Tn5 contains two internal *Hind*III sites²², *Hind*III digestion of pRiA4b::Tn5 from R1023 generated two new *Hind*III fragments containing the right and left portions of the wild-type H-30a fragment (2.0 and 2.25 kb, respectively), plus a Tn5 *Hind*III internal fragment (3.5 kb). Hybridization of λ Ng31 to *Hind*III digested DNA from R1023 demonstrated that this insertion occurred within the cT-DNA-homologous region, as both the 2.0 and 2.25 kb mutant fragments were detected (Fig. 4, lane 4). Thus, at least one genetic locus can be assigned to the cT-DNA region of the Ri plasmid. More information concerning the presence and expression of any corresponding genes of the plant cT-DNA must await transcriptional and sequencing studies.

The evidence presented here suggests a close evolutionary relationship between a region of the Ri plasmid and a region of the normal *N. glauca* genome. This homology was detected in conditions of hybridization that allow approximately 12% base-pair mismatching. Homology between various *Agrobacterium* T-DNA sequences and sequences from a variety of plants may be found as more Ti plasmids are examined. Some of these homologous regions may not be detected until less stringent conditions of hybridization are used, or until functionally related proteins are discovered and the amino acid sequences determined. The origin of the cT-DNA remains unknown. Perhaps in the evolution of *Agrobacterium*-plant relationships, the bacterium has captured plant genes, which it then reinserts into the plant genome, analogous to the acquisition of host oncogenes by some RNA tumour viruses²³. Alternatively, plants may have sequences acquired from past infections by *Agrobacterium*. It is also possible that Ri and Ti plasmids have evolved from a common progenitor of sequences present in higher plants. We might infer from the mutational analysis of the plasmid sequences that a genetic locus, perhaps involved in the control of plant growth, is located on the corresponding plant sequence.

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Does Q β replicase synthesize RNA in the absence of template?

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Q β replicase, in the absence of added template, will synthesize RNA autocatalytically¹. A variety of small RNA species, termed '6S RNAs' are generated². As this reaction purportedly occurs in the absence of template, it has been termed 'de novo' RNA synthesis³. The question of whether Q β replicase can polymerize replicatable RNA molecules, without instruction from a template, has important evolutionary implications. The finding that Q β replicase was able to synthesize RNA *de novo* was based on (1) failure to find contaminating RNA in Q β replicase preparations³; (2) differences in the sizes of products of apparently identical reactions⁴; and (3) kinetic differences between template-instructed and *de novo* reactions⁵. Here we describe a procedure for production of Q β replicase lacking one of its subunits, ribosomal protein S1, involving column chromatography in the presence of a low concentration of urea. We show that the resulting highly purified enzyme will not synthesize detectable RNA in the absence of added template. We show also that the ability to perform a reaction kinetically indistinguishable from the *de novo* synthesis reaction can be restored to the highly purified enzyme by adding a heat-stable, alkali-labile component of Q β replicase preparations. Thus our findings suggest that, in the *de novo* reaction, Q β replicase is replicating previously undetected contaminating RNA molecules.

In the novel procedure used here, Q β replicase is applied to phosphocellulose P-11 and is then washed with buffer containing 2 M urea, which releases the S1 from the bound replicase. The remaining enzyme subunits are eluted with a high salt

buffer lacking urea. This enzyme, termed R(-S1), is completely free of detectable S1, as determined by silver-stained SDS-gel electrophoresis. As for R(-S1) made by other techniques^{3,6,7} it shows normal activity with synthetic templates containing cytidylate but is inactive with Q β RNA in standard conditions (data not shown). However, this enzyme also will not synthesize RNA in the absence of added template (see below).

We found that several different Q β replicase preparations were able to synthesize RNA in the absence of exogenously added template. The characteristics of this RNA synthesis were similar to those described previously for reactions in which no template was added^{4,5}: after an initial period during which no synthesis could be detected, incorporation of ³²P-UMP occurred for several hours (Fig. 1). The length of the lag period was inversely proportional to the concentration of nucleoside triphosphates. Also, no RNA was synthesized at NTP concentrations <0.15 mM (data not shown)⁵. The products of this

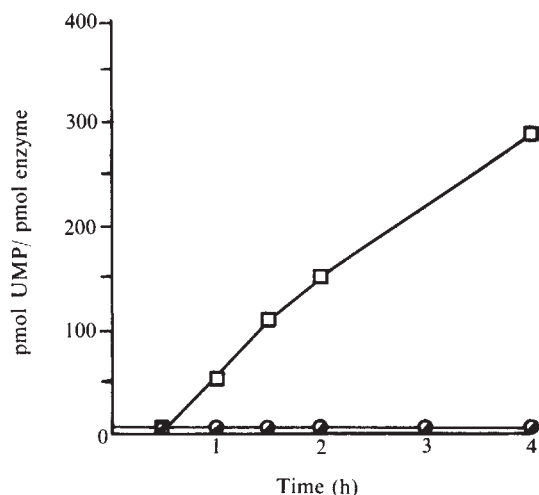


Fig. 1 Time course of template-free synthesis by Q β replicase and R(-S1). The final enzyme concentrations were: $\square$, 63 nM replicase; $\circ$, 68 nM R(-S1); and $\bullet$, 68 nM R(-S1) plus 82 nM S1.

Methods: Q β replicase was purified as described elsewhere⁹. R(-S1) was purified by the following procedure. Q β replicase was dialysed against 50 mM Tris-HCl pH 7.5, 1 mM EDTA (TE), containing 0.2 M KCl, loaded at 1/2 column volume per h onto a 0.9 $\times$ 3.0 cm phosphocellulose P-11 column (PC column) previously equilibrated in dialysis buffer. The column was washed with 10 ml of TE, 0.2 M KCl followed by 10 ml of TE, 0.2 M KCl containing 2 M urea. The urea was removed by washing the column with 8 ml of the original buffer. The enzyme activity was step-eluted with TE, 0.4 M KCl. Fractions (1 ml) were collected and 10- μ l aliquots were assayed for poly(C)-dependent poly(G) polymerase activity⁹. Active fractions were pooled and dialysed against TE, 20% w/v glycerol, 0.175 M NaCl and loaded onto a 1 ml DEAE-Sephadex A-50 column equilibrated in dialysis buffer. The DEAE column was washed with 2.5 ml of the same buffer and step-eluted with 5 ml of the same buffer plus 0.3 M NaCl. Fractions (0.5 ml) were collected and 10- μ l aliquots were assayed for activity as before. The location of the R(-S1) fractions was determined by silver staining¹⁰ of SDS-polyacrylamide gels¹¹. The R(-S1) fractions were pooled, and the ionic strength adjusted to 0.175 M NaCl. The R(-S1) was then loaded onto a 1 ml PC column equilibrated with TE, 20% w/v glycerol and 0.175 M NaCl. The column was washed with 5 ml of the same buffer, then R(-S1) was step-eluted by washing the column with 10 ml of TE, 20% w/v glycerol, 0.4 M NaCl. Active fractions were pooled, dialysed against TE, 20% glycerol, 0.1 M NaCl, and stored at -20 °C. S1 (given by G. Carmichael) was purified according to a procedure described elsewhere¹². Q β replicase, R(-S1) plus S1, and R(-S1) were assayed for template-free synthesis at 30 °C in a 200- μ l reaction containing 500 μ M [α -³²P]UTP (55 μ Ci ml⁻¹) according to the procedure of Sumper and Luce³. At the indicated times, 20- μ l aliquots were withdrawn and assayed for TCA-precipitable material.

'template-free' RNA synthesis were analysed by gel electrophoresis (Fig. 2, lanes 6, 7); many small RNA species were found. No two reactions were identical, even with multiple aliquots of the same enzyme preparation. However, greater differences were evident when samples from different enzyme preparations were compared (unpublished observations).

When we tested the R(-S1) for *de novo* RNA synthetic activity, no incorporation of ³²P-UMP was detected (Fig. 1; Fig. 2, lane 3). Furthermore, no RNA synthesis was detected after addition of equimolar amounts of native S1 to the R(-S1) (Fig. 1). In a control experiment we found that added S1 does allow R(-S1) to replicate Q β RNA (not shown).

We hypothesized that the urea-phosphocellulose column removed previously undetected small RNAs from the replicase together with the S1. To test this, we performed the following experiment: a sample of Q β replicase, capable of 'template-free' synthesis, was heated to 90 °C for 10 min and then used to supplement R(-S1) in the template-free synthesis reaction. The heat-treated replicase was incapable of template-free synthesis (Fig. 3; Fig. 2, lane 2). Presumably the enzyme was irreversibly denatured, as it was also incapable of transcription of poly(C) (data not shown). Although the R(-S1) alone synthesized no RNA, the R(-S1) plus heat-inactivated replicase synthesized RNA with kinetics similar to untreated control

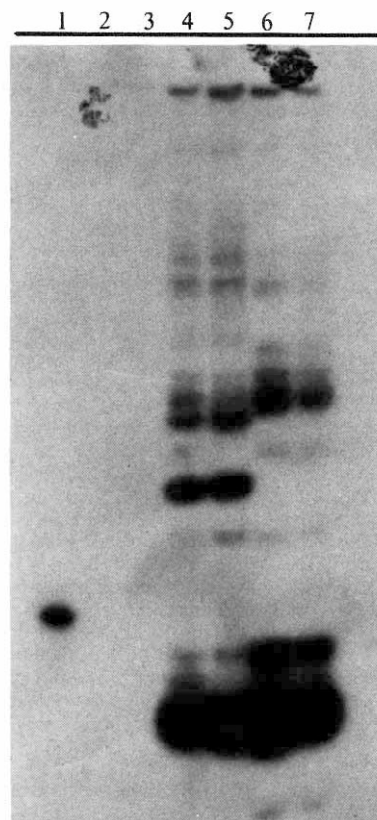


Fig. 2 Analysis of the products synthesized in a template-free reaction by Q β replicase and R(-S1). After 90 min and 24 h incubation, 10 μ l aliquots of each reaction described in Fig. 3 were removed, phenol-extracted, and prepared for electrophoresis as described elsewhere¹³. The reaction products were electrophoresed on an 8% polyacrylamide-7 M urea gel for 4 h at 600 V (ref. 13). RNA 1 (110 nucleotides), the negative controlling element of plasmid ColE1 replication¹⁴, was electrophoresed as a marker. The samples shown on the autoradiogram are: lane 1, RNA 1; lane 2, 24-h time point of the heat-inactivated replicase reaction; 3, 24-h time point of the R(-S1) reaction; lanes 4 and 5, 90-min and 24-h time points of the R(-S1) plus heat-inactivated replicase reactions, respectively; lanes 6 and 7, 24-h and 90-min time points of the replicase reactions, respectively.

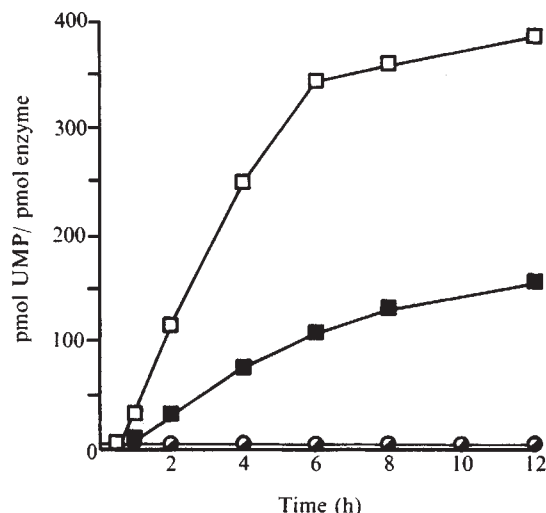


Fig. 3 Time course of template-free synthesis by R(-S1) supplemented with heat-inactivated Q β replicase. Template-free synthesis by Q β replicase and R(-S1) was assayed at 30 °C in 100 μ l of the mixture described in Fig. 1 legend. At the indicated times, 10- μ l aliquots were withdrawn and assayed for TCA-precipitable material. For reactions with heat-inactivated Q β replicase, 3 μ g of replicase was first heated at 90 °C for 5 min. Final enzyme concentrations were: $\square$, 33 nM untreated Q β replicase; $\circ$, 66 nM heat-inactivated replicase; $\bullet$, 68 nM R(-S1); and $\blacksquare$, 68 nM R(-S1) plus 66 nM heat-inactivated replicase.

Table 1 Effect of Q β replicase inactivation on 'template-free' synthesis

	Incorporation (pmol) per pmol enzyme
Q β replicase	61.4
Q β replicase (90 °C)	0.5
R(-S1)	0.5
R(-S1)+Q β replicase (90 °C)	13.7
R(-S1)+Q β replicase (90 °C, pH 9)	0.4
R(-S1)+Q β replicase (90 °C, DNase)	8.9

Template-free synthesis by replicase and R(-S1) was assayed at 30 °C in 75- μ l reactions containing 500 μ M [α - 32 P]UTP (27 μ Ci ml $^{-1}$) as described in Fig. 1 legend. After 8 h incubation, aliquots (15 μ l) were removed and trichloroacetic acid (TCA)-precipitable material was determined. Q β replicase was present in all template-free reactions at a concentration of 29 nM. Where indicated, R(-S1) was present at a concentration of 68 nM. 1 μ g of Q β replicase was heated to 90 °C for 5 min in the presence of alkali (1 mM EDTA brought to pH 9.0 with 1 M NaOH, followed by neutralization with 1 M HCl). In the control, equivalent amounts of NaOH plus HCl were added simultaneously. 1 μ g of Q β replicase was also heat-inactivated, then incubated for 5 min at 37 °C in the presence of 0.1 μ g of Worthington RNase-free DNase before the onset of template-free synthesis.

enzyme (Fig. 3). Furthermore, the major products of the reactions catalysed by control replicase and by the R(-S1) plus heat-treated replicase were similar (Fig. 2, lanes 4, 5). We heat-treated several other replicase preparations and tested them for ability to promote template-free synthesis by R(-S1). In each case they were able to do so, and in each case the pattern of RNA synthesis resembled (but was not identical to) the pattern found with that replicase before boiling. These results suggest that the replicase preparations contain small amounts of 6S RNA species which can be replicated by Q β replicase.

The observation that the factor which enables R(-S1) to synthesize RNA is resistant to a 90 °C heat treatment suggests that it is not a protein. It is probably a small fragment of nucleic acid contaminating the Q β replicase preparations. To test the possibility that the factor is RNA, we treated a sample of Q β

replicase at 90 °C at pH 9.0 for 10 min and then neutralized the solution. When this alkali-treated replicase was mixed with R(-S1), no synthesis was observed (Table 1). On the other hand, replicase which was heat-treated and then treated with DNase did not lose its ability to stimulate template-free RNA synthesis by R(-S1) (Table 1). These experiments indicate that the contaminating factor which allows R(-S1) to synthesize RNA *de novo* is RNA—we believe that this RNA is probably serving as a template.

To prove that the component supplied by the boiled replicase is RNA, it would be necessary to show that it is eliminated by RNase. As the product of the reaction is also RNA, an RNase that can be inactivated before RNA synthesis is initiated such as micrococcal nuclease, must be used. We found that treatment of the boiled replicase with micrococcal nuclease followed by inactivation of the nuclease with EGTA before RNA synthesis did not inactivate the heat-stable factor, but it did reduce rather the size of the RNAs synthesized in the template-free reaction (data not shown). We also found that the product RNA was not made acid-soluble even by extensive micrococcal nuclease treatment. Thus, this experiment is consistent with the idea that the heat-stable component is RNA, and that this RNA serves as a template. However, both the template and product of this reaction are resistant to the action of micrococcal nuclease.

Two hypotheses have been proposed for the origin of the 6S RNAs observed after template-free synthesis. The first is that an RNA template is produced *de novo* by replicase and this template is then replicated autocatalytically³. In this hypothesis the lag time associated with template-free synthesis represents the time required for replicase to assemble suitable templates from precursors and then replicate them to observable levels^{4,5}. The second hypothesis is that traces of 6S RNA contaminate even highly purified replicase preparations and these RNAs serve as templates for autocatalytic synthesis^{2,8}. In this hypothesis the lag time represents the time required for replicase to generate suitable, autocatalytically replicating templates through errors in transcription of the endogenous RNA molecules. Neither product analysis nor kinetic analysis is capable of unequivocally demonstrating which of these hypotheses is correct.

Here we have described a novel procedure, involving chromatography in the presence of a denaturing agent (2 M urea), for the production of Q β replicase lacking a subunit (S1). The resulting enzyme was the purest we had ever obtained: it contained no contaminating polypeptides based on silver-stained SDS-gels. Apparently the procedure also removed previously undetected contaminating RNA molecules as no RNA was synthesized in the absence of added template. This discovery provided us with the first functional assay for the presence of contaminating RNA in Q β replicase preparations. Using this assay we demonstrated that our Q β replicase preparations capable of *de novo* RNA synthesis contained a heat-stable alkali-labile component which enabled the highly purified enzyme to perform a reaction kinetically indistinguishable from the *de novo* reaction.

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In the ghetto

David Cohen

THE Society for Psychical Research was set up in 1882 by eminent Victorian scientists who believed that, with the proper attention and the right instruments, there would be scientific answers to the ultimate questions. Does telepathy exist? Can mind move matter? Are there ghosts? And, the most profound of mysteries, do we survive after death? The Victorians were confident that, with the correct methods, such questions could be resolved. The Society was then more evangelical than it is now and, by 1891, it had to warn members against complacency. A circular reminded them to continue to log all apparitions and manifestations; not everyone, it seems, was convinced by the evidence for life after death.

Throughout its one hundred years, the Society has attracted many distinguished scientists. Early presidents included the physicist Sir William Crookes, the psychologist William James, and Henri Bergson. I suspect that many of these founding fathers — women played no part as investigators but were, of course, often investigated as mediums — would be bitterly disappointed to learn that a century later there are still no definite answers and still no certitudes. Controversy reigns yet.

Ivor Grattan-Guinness has assembled a rather bitty book to celebrate the centenary of the Society. There are 34 chapters written by 27 different authors — assuming that the chapters written by “Brian Nisbet” and “the late Brian Nisbet” are both the product of the same earthbound pen. If not, I think we should be told how the manuscript got through.

Certainly, the book leaves little out from astrology to ufology. There are chapters on telepathy, metal bending, psychic healing, ghosts, teaching psychic research and much else besides. In theory, this should make a comprehensive, authoritative guide; in practice, however, it does not. With so many different authors, there is no unifying perspective. Each contributor is an enthusiast for his, or her, brand of psychic experience. We learn little about how various fields of psychic research relate; for instance, if there is such a phenomenon as telepathy, does that imply anything about ghosts?

Nevertheless, there are good chapters. Hans Bender gives a lucid account of the state of the poltergeist. He has impressive evidence from the Rosenheim case where many sober German engineers measured strange forces in a house where bulbs exploded “spontaneously” and things did go bump — day and night. Susan Black-

Psychical Research: A Guide to its History, Principles and Practices. Edited by Ivor Grattan-Guinness. Pp.424. ISBN 0-85030-316-8. (Aquarian Press, Northamptonshire: 1982.) £7.95, \$14.95.

more writes interestingly about out-of-the-body experiences and argues that we should link them to our less-aerial imaginative experiences. Many contributions, however, are patchy, such as those on telepathy and clairvoyance and on survival after death. This is particularly disappointing because the latter subject keeps cropping up as a key idea.

Wide-ranging though it seems to be, the book fails to ask some important questions. Why, for example, are people so interested in the paranormal? To a sceptic like myself, it seems that a powerful motive for doing psychic research is to prove that there is an after-life and that you, the researcher, will get to enjoy it. There is also little attempt to weigh up the evidence

selectively and to suggest that, perhaps, the evidence for some paranormal claims is much stronger than for others.

The other great weakness is that the book barely explores the relationship between parascience and mainstream science. Most paranormal research is reported in journals devoted exclusively to the subject. It all remains outside “normal” science. That is due partly, of course, to the dogmas of “normal” science which often regards such weird wonders necessarily as nonsense. But to create a ghetto for parascience is not healthy. It allows its practitioners to get away with much poor thinking and methodology, and it also allows normal science to evade the challenge posed by impressive evidence such as that in the Rosenheim case and in some telepathy results. This book is an affectionate tribute to a worthy Society but is not the authority it aspires to be. □

David Cohen is Editor of *Psychology News*.

Turning over puzzles in the mind

Stuart Sutherland

Mental Images and their Transformations. By Roger N. Shepard and Lynn A. Cooper. Pp.364. ISBN 0-262-19200-4. (MIT Press/Bradford: 1982.) \$25, £20.

REVERTING to an earlier tradition, psychologists are becoming increasingly preoccupied with the mind. They are even using introspection as a source of ideas about mental activity, but unlike their predecessors who worked in the early years of this century they feel that before being made public the results of introspection must acquire the dignity that only experiments can lend.

Professor Shepard is one of the most cunning masters of this technique. In 1971 he reported an experiment in which subjects were shown pairs of shapes of the kind displayed in Fig. 1 (see p.354). The members of each pair were either identical or were mirror images of one another. The two shapes were shown at different orientations, one being rotated with respect to the other through an angle that varied from 0 to 180 degrees. The task was to decide whether the two shapes were identical. Shepard found that the time taken to reach a decision increased linearly with the angular difference in orientation between the members of the pair being

viewed and that the increase was the same whether one shape was rotated with respect to the other in the frontal parallel plane or around a vertical axis, that is to say, rotated in depth.

Shepard concluded that visual images can be rotated in the head and that it takes a constant time to rotate an image through a constant angle. He and his colleagues corroborated this result in a series of ingenious experiments. They found, for example, that if subjects were told to rotate a given shape in advance through a particular angle and were then presented with a test shape, the time taken to decide whether it was the same as the original shape was determined by the difference in the orientation of the test shape and the orientation to which the subjects had mentally rotated the original. Shepard was also able to demonstrate that when a shape is being mentally rotated through a given angle, the image passes through the intermediate orientations. Of course his subjects could — and did — tell him how they were performing the task, but it required experiments to show that the rate of rotation was constant per unit angle.

Mental Images and Their Transformations, written in collaboration with Dr Cooper, contains little that is new. Most of

the book comprises 12 experimental articles already published elsewhere; although some of them have been pruned, there is considerable repetition of material. Nevertheless, it is useful to have these articles brought together and the writing is always clear, sometimes elegant. Shepard and Cooper have added four new chapters in which they relate their work to that of others and expound some of the theoretical issues. It is a pity they did not decide to give a more thorough review of the whole subject: while the accounts of their own experiments are unnecessarily detailed, their accounts of those of others are too elliptical and the reader will have to go the original sources to discover exactly what has been found out.

Work on mental rotation has given rise to two controversies, one experimental, the other theoretical. The main experimental issue is whether the speed of mental rotation is constant regardless of the complexity of the shape. Shepard allows that the time may depend on the familiarity of the shape. He has also found that when two identical three-dimensional shapes in different orientations are projected in succession on the eye, the observer involuntarily sees a continuous rotation of

the shape from one orientation to the other, and that the time for rotation through a unit angle is much less than when a mental image of a shape is deliberately rotated. He maintains, however, on the basis of his own experiments that complexity does not affect rotation time. Other experiments indicate that it does. Moreover, introspection suggests that mentally rotating a complex image like that of a face while preserving all the details is more time consuming than rotating the image of a line. Indeed, no matter how much care is taken, many of the details of the face tend to dissolve in the act of rotation.

Of more importance is the theoretical dispute, which has to do with the nature of the brain's representation of the visual world. Shepard argues that his experiments show that this representation is an analogue one. An alternative form of representation is a structural description, in which the object is broken down into parts, and the structure of each part and the relation between the parts are specified in terms of lower level entities, like lines and edges, and relationships, like "above" and "to the right of". Descriptions of this sort are called propositional because they contain entities, relations and properties, though the representation of these elements is not of course verbal.

It is hard to reconcile the opposing sides in this dispute. On the one hand, it is difficult to build a theory of object recognition on an analogical representation and both introspection and experiment suggest that vision breaks objects into parts. It is, moreover, extremely hard to envisage how an analogue description could be stored let alone rotated in the nervous system. On the other hand, Shepard's results, annoying though they are, will not go away. It may be that we can only perform operations on propositional descriptions that correspond to the ways in which objects can be transformed in the real world. Since a real object cannot rotate through a given angle except by passing through intermediate angles, it may be that the transforms of a propositional description that correspond to rotation can only be performed in small (though not necessarily continuous) steps.

It is at this point that the experimental and theoretical disputes converge. In a propositional description, it is unlikely that all the elements could be rotated at once, because one can only keep before the mind a limited number of elements at any one moment of time. It follows that if mental descriptions of visual objects are propositional, complex objects should take longer to rotate than simple ones. *Pace* Shepard, it would make life easier for visual theorists if this does turn out to be the case. The problem of imagining a face upside down gives one hope. □

Stuart Sutherland is Director of the Centre for Research on Perception and Cognition at the University of Sussex.

Hunt the scientist

Howard J. Lewis

American Men and Women of Science, Physical and Biological Sciences, 15th Edn. Seven volumes, pp.7,010. ISBN 0-8352-1413-3. (Bowker: 1982.) \$643.50, £357.50.

IN the opening statement, the author of the editorial preface to this new edition of *American Men and Women of Science* goes a bit too far: "AMWS is without peer as a chronicle of North American scientific endeavor and achievement". This set of books may have a far larger cast than, say, the chronicles of Holinshed, but as an account of achievement it is short on drama. It also lacks some authority with respect to its geographical scope: there is no one on its Advisory Committee of 16 from outside the United States. On the other hand, the type is more legible than before, and the paper is gratifyingly opaque. But it is still heavy: the seven-volume fifteenth weighed out at 26 pounds on the bathroom scale.

AMWS has earned its pre-emptive position in this corner of publishing as a reasonably accurate *Who's Who* of American science. It also serves to identify potential candidates for faculty positions, committee appointments and the like; but for this sort of information no longer is it necessary to search through tedious disciplinary and geographical indexes. They have been jettisoned in favour of a promised service of on-line searches through Bibliographical Retrieval Services, Inc., DIALOG Information Services and the Jaques Cattell Press. "Promised", because at the time of writing neither BRS or DIALOG is yet on line. That capability is now expected in a matter of a few months, not — as a publication announcement declares — by October 1982. Directories are evidently a dicey business: the fourteenth edition promised a follow-on directory of the behavioural and social sciences that never materialized.

When on-line search is available, it will permit subscribers to riffle electronically through the 130,000 men and women in the fifteenth edition. All the basic elements in the individual biographies will be accessible — earned degrees, research speciality, alma mater, awards, professional affiliations, geographical location and so on. Thus if one has a sudden need to identify a Midwestern holder of a PhD who has carried out research on the Onagraceae and Solanaceae, Professor John E. Averett will be unearthed, like a truffle, at the University of Missouri in St Louis.

The contents of the fifteenth edition are characterized by the editors as "profiles [of] living scientists in the physical and biological fields, as well as public health scientists, engineers, mathematicians, statisticians, and computer scientists". How complete is it? Checked against a sample of the distinguished members of the

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Fig. 1. Examples of pairs of shapes presented to subjects in experiments on mental rotation. (a) A "same" pair, which differs by an 80° rotation in the picture plane; (b) a "same" pair, which differs by an 80° rotation in depth; and (c) a "different" pair, which cannot be brought into congruence by any rotation. (From "Mental Rotation of Three-Dimensional Objects" by R.N. Shepard and J. Metzler *Science* 171, 702; 1971.)

National Academy of Sciences, it does excellently; against the equally distinguished members of the National Academy of Engineering, not so well but still respectably.

How it copes with the newer recruits to the life of science is yet another question. The fourteenth edition added 13,000 new names to the roster; the fifteenth only 7,500. According to the editors, there were fewer nominations this time, and only 20 per cent of the forms sent out to the nominees were returned, a figure that seems astonishingly low. All those returned were reviewed for acceptability, 75 per cent of them being considered suitable for mention in the new edition.

A random test of the effectiveness of updating suggests that it has been spotty. Some very recent moves have been caught by the editors, others missed. David Hamburg, who in October 1980 left the presidency of the Institute of Medicine in Washington DC to join the Harvard faculty, is still listed at the Institute, but oddly with the Harvard address. By the time the fifteenth edition reaches most library shelves, Dr Hamburg will already have left Harvard to become president of the Carnegie Corporation of New York. Such are the woes of the editors of AMWS.

Howard J. Lewis, based in Washington DC, is a writer on science policy.

EGG and malignancy

Peter Alexander

The Biology of Tumour Malignancy. By G.V. Sherbet. Pp.255. ISBN 0-12-639880-1. (Academic: 1982.) £19.20, \$36.

UNDERSTANDING of the nature of malignant transformation at the molecular level is advancing at a pace which is truly phenomenal. Important new findings appear almost every week. The outline of a general theory is clearly emerging and this seems to account adequately for many of the findings in the field of carcinogenesis. The hypothesis that the malignant phenotype arises from a quantitative and not a qualitative change in the genome, such that

one or more of a group of diverse normal genes — the oncogenes — are expressed to a greater extent, is gaining support all the time.

So far so good, but cancer as a disease has to be considered in the context of the whole organism. The full potential of new approaches to both treatment and prevention, which derive from the currently acquired knowledge of the sub-cellular lesions responsible for cancer, is unlikely to be realized without a better understanding of the role of the host which can determine the pattern and rate of growth of cells that have been transformed. Both seed and soil need to be considered.

Quite excellent monographs dealing with the interaction of host and tumour have been written by Rupert Willis and Leslie Foulds, and these are still essential reading for the cancer researcher. But they are now dated and an up-to-date treatise would be immensely valuable. The slim book of Dr Sherbet does not, unfortunately, fill this role as its principal objective is to describe a test for assaying the malignancy of a tumour from a biopsy specimen by a procedure developed by the author and called "epigenetic grading" (EGG).

In the test, cells from the tumour are injected into embryonic tissue of the chicken — the 18-hour old blastoderm — cultured together for some 18 hours and then examined histologically. According to the author, the morphological response of the embryonic tissue to the tumour cells correlates with the malignancy (here defined as invasiveness and capacity to metastasize) of the tumour. The data to substantiate the claim made for EGG, are, however, extremely thin and the scientific basis of the technique remains imprecise. This topic may be of interest as a research project but has not advanced sufficiently to justify the writing of a monograph.

The first third of the book provides a thoughtful, well-informed if brief and therefore selective review of the biology of metastasis; cell detachment, invasion, distant seeding and the part of the immune system in these processes are considered in an orthodox frame. While this chapter is a useful introduction to the topic, it is not enough to warrant the publication and purchase of the entire book. □

Peter Alexander, formerly at the Institute of Cancer Research, is now in the CRC Medical Oncology Unit at the General Hospital, Southampton.

No direct path from genes to phenotype

Lindon J. Eaves

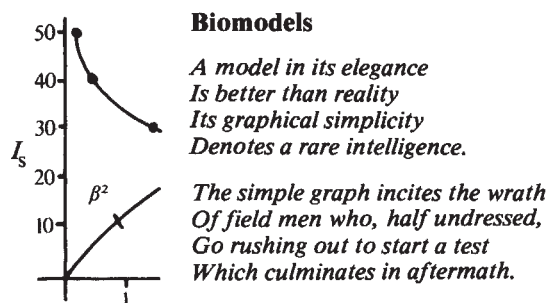
Biometrical Genetics: The Study of Continuous Variation, 3rd Edn. By Kenneth Mather and John L. Jinks. Pp.396. ISBN 0-412-22890-4. (Chapman & Hall: 1982.) £22.50, \$49.95.

ABOUT 80 years ago, Karl Pearson browbeat William Bateson and his fellow Mendelians for their mathematical incompetence. The image of quantitative genetics has never recovered from this early conflict between mathematics and biology. Many geneticists still view biometrical genetics as an arcane pursuit, one that has been eclipsed by developments in molecular genetics. Mather and Jinks, founders of what many now describe as the "Birmingham school" of biometrical genetics, are almost unique in being geneticists, rather than mathematicians, who have applied their talent to understanding the origin and significance of continuous variation.

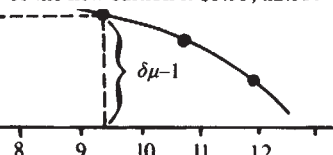
Readers of this new edition of Mather and Jinks should be left in no doubt that biometrical genetics addresses fundamental biological issues that are left untouched by the molecular approach. Variation in continuous traits is seldom entirely genetic and rarely due to just one or two loci. Furthermore, genes often do not contribute additively to the phenotype but may interact with one another and with the environment, and may even create the environment in which they are expressed. Theories of the origin and evolutionary dynamics of variation will fail if they are developed in ignorance of the rules relating effects of individual genes at the molecular and biochemical level to the complex aspects of the phenotype on which natural and artificial selection are based. This perspective is essential to the scientific understanding of variation within species.

The book describes how the statistics derived from breeding studies can be interpreted in genetic terms and how studies can be designed to enhance the power of a genetic analysis. The treatment of genotype-environment interaction, for example, goes far beyond a mere statistical description of interaction and into the analysis of gene action affecting sensitivity to the environment. Mather and Jinks offer breeding programmes to resolve the additive, dominant and epistatic components of gene action and the effects of linkage and maternal inheritance; in particular, their discussion of the "effective factor" should lend caution to the current quest for single loci of large effect. They present data showing that estimates of the number of genes affecting a quantitative trait are directly proportional to the industry and ingenuity of the investigator.

Critics have commented that Mather and



"Biomodels" is taken from John M. Burns' collection of poems, *BioGraffiti: A Natural Selection*, which has recently been re-issued in paperback by W.W. Norton. Price of the new edition is \$3.95, £2.95.



Jinks write too much about inbred lines and their own data, but in this book their analysis gives equal weight to studies of inbred lines and randomly mating populations. Few programmes have explored the genetics of quantitative variation with such precision as that in Birmingham. The species selected for study have been especially amenable to genetic manipulation through breeding, randomization was conducted at the level of individual plants rather than plots and, above all, the authors have data on the same material going back over 30 years in many cases.

Researchers in genetics and the social sciences will discover that the genetic basis of individual differences is more complex than is presupposed in many models for human variation. The strength of the approach to variation developed by Mather and Jinks lies in representing individual genes algebraically and tracing their cumulative and interactive effects on a continuous phenotype. The models extend readily to include genotype-environment interaction and the effects of genes on environment which are often discounted arbitrarily in human studies. In many respects, therefore, Mather and Jinks pre-empt much of the recent work on non-genetic transmission. One factor, however, prevents the whole-hearted application of the biometrical approach to human populations. The models they develop begin at the genotype and work

outwards. The approach is powerful when the genetic structure of a population can be manipulated by breeding and when interactions between phenotypes can be minimized by randomization. In human populations, however, the consequences of assortative mating and cultural inheritance may depend on social interactions at the phenotypic level and are difficult to represent by the cumulative finite effects of many discrete loci. For this reason, the treatment of assortative mating requires distinct methods which have still to be reconciled with the biometrical approach. Similarly, although Mather and Jinks trace the environmental consequences of genetic differences such as those of the maternal genotype, models of family resemblance for cultural traits ultimately require series which do not end at the parental genotype.

Although the book will help others design, analyse and interpret their own experiments, it is not just a "cookbook". It presents the single most compelling body of evidence to remind all who study variation in plants, animals or man that genetic effects on continuous variation are inherently subtle and that the failure of studies to resolve them says more about basic limitations in the design and analysis of investigations than the underlying simplicity of genetic effects.

The significance of the book lies in the rigorous model-building and data collection which lie behind the authors' attempt to analyse continuous variation. Mather and Jinks's own thorough statement of the theory of multifactorial inheritance and its implications for the analysis of phenotypic variation is based on two generations of painstaking work. Such experience cannot be won cheaply. The opportunity to share the authors' insight will be the lasting value of their work for any investigator of the new generation with the stamina to follow it. □

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Of course, the accuracy of a palaeogeographical map depends to a large extent on the nicety of regional geological correlation and the quality of information available. The data points provided — or implied — on a such a map are like the data points on a graph. They allow the reader to assess the validity of the interpretation provided by the map itself.

The *Geological Atlas of Western and Central Europe* provides an overview of the tectonic and stratigraphic framework and a summary of European evolution from the Late Silurian onwards, a period of about 400 Myr. It is a lavishly illustrated *tour de force* and Peter Ziegler is to be congratulated on the production and presentation of a fine working document. Shell too deserve considerable praise in permitting the release of their collected regional data. The maps (40 full-colour enclosures) and 29 line drawings provide thought-provoking frames of reference for the exploration geologist and academic stratigrapher alike.

In a work of this breadth there are inevitably points of criticism. The maps, for instance, are largely devoid of data points, it being suggested that this is because of confidentiality and the constraints of space. However, two transparent overlays giving the outline of Europe are provided and it would have been a relatively simple task to locate released wells on these.

The text is a narrative to the coloured maps, but as such it does not provide sufficient basic information. Thus speculations embodying vastly broad concepts (e.g. convergence and collision in trench-arc systems) are sometimes left as *ex cathedra* statements unqualified by even a supporting source reference. (To be fair, however, there are over 650 references, all essential, and so the book provides a valuable bibliographic overview of European evolution.) I also found the time-frame of some maps misleading. Instead of providing maps over as precise as possible a time-period, some have been needlessly generalized. This is particularly true within the Mesozoic where, for example, one map covers the bulk of the Lower and part of the Middle Jurassic (a 27 Myr period), another encompasses the entire Upper Jurassic (a 19 Myr period) and yet another embodies the Upper Cretaceous to lowest Tertiary (about 37.3 Myr). Such maps are confusing at best and misleading at worst, the equivalent of attempting to capture all England's Tudor monarchs in one single photofit image!

Notwithstanding these criticisms the two-volume compilation embodies a colossal labour by a geologist of great talent. The *Geological Atlas* will be a valuable and provocative base for future research — and it comes at a very reasonable price. □

Bruce Sellwood is Lecturer in Geology at the University of Reading.

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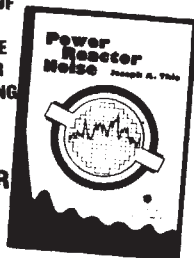
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Being sure of Shell

Bruce Sellwood

Geological Atlas of Western and Central Europe. By Peter A. Ziegler. Pp.130. (Elsevier Scientific: 1982.) Dfl.160, \$68.

AS WELL as being tremendous fun to draw, palaeogeographical maps are essential to the exploration geologist. Such maps aid the delineation of sedimentary basins and are a vital tool to the extractive geological industries. In addition to the former position of shore lines, such maps should also show the nature of the deposited sediment within the basin and its thickness.

Appointments

Sir John Kendrew, president of St John's College, Oxford, UK, and Nobel prize-winner for chemistry in 1962, has been elected chairman of the Governing Council of the United Nations University.

Professor Sir Hans Kornberg has been elected to be the 34th Master of Christ's College, Cambridge, UK since the college's foundation in 1504. Sir Hans succeeds Sir John Plumb who retired in July.

Sir Alistair Pilkington has been appointed Chairman designate of the Council for National Academic Awards, UK. He will succeed Sir Denis Rooke. **Professor J.M. Ziman**, Visiting Professor in the Department of Social and Economic Studies, has been appointed as a member of the Council.

Dr Brian Fender, a chemist and neutron scientist from the University of Oxford, has been appointed director of the Institut Max von Laue-Paul Langevin, a research establishment at Grenoble, France, funded jointly by the United Kingdom, France and Germany.

The title of Honorary Professor at Bristol University, UK, has been conferred upon **Professor Colin Andrew** following his resignation from his university chair in the Department of Mechanical Engineering. Also, **Professor J.E. Enderby** has been appointed to the H.O. Wills Chair of Physics for the duration of his term of office as head of the Department of Physics.

Dr Peter J. Lewis has been named director of the Centre de Recherche Merrell in Strasbourg, France.

Derek Pugh, professor of organizational behaviour at the London Business School, has been appointed to the Chair of Systems at the British Open University.

The College of Physicians and Surgeons of Columbia University has created a new centre and an endowed professorship for the study of social issues in medicine. **David Rothamn**, professor of history at Columbia and a renowned social historian, has been appointed director of the Centre for the Study of Society and Medicine as well as the first Bernard Schoenberg professor of social medicine.

The Australian Atomic Energy Commission, (AAEC) has announced the appointment of **Dr D.G. Walker** as its Chief Executive Officer and Director of the AAEC Research Establishment, Lucas Heights.

Wallace Behnke, vice chairman of the board of Commonwealth Edison Company and a prominent engineer in the nuclear field, has been elected chairman and chief executive officer of the American Atomic Industrial Forum, an international association of approximately 600 organizations interested in the peaceful uses of nuclear energy.

Mr William J. Dircks, executive director for operations of the U.S. Nuclear Regulatory Commission, has been elected chairman of the Committee on the Safety of Nuclear Installations (CSNI), one of six standing committees of the OECD Nuclear Energy Agency (NEA).

Awards

The 1982 Albert Lasker Medical Research Award of \$15,000 will be shared by **Dr Michael Bishop** and **Dr Harold Varmus** of the University of California at San Francisco, **Dr Hidesaburo Hanafusa** of the Rockefeller University, **Dr Raymond Erikson** of Harvard University and **Dr Robert Gallo**, head of the Tumour Cell Biology Laboratory at the National Cancer Institute. The prize is given for contributions that have significantly increased understanding of the mechanisms of cancer at the molecular level.

The first Behring Metchnikoff immunology prize has been awarded to **Mme Pierrette Chateaufreynaud-Duprat** and **M Philippe Roch**.

Professor Glenn Seaborg, associate director of the Lawrence Berkeley Laboratory, and professor of chemistry at the University of California at Berkeley, has been awarded the Henry DeWolf Smyth Nuclear Statesman Award by the Atomic Industrial Forum and the American Nuclear Society.

The United Nations Food and Agricultural Organisation has awarded an André Mayer Research Fellowship to **Dr Malcolm Beveridge**, of the University of Stirling's Institute of Aquaculture. Dr Beveridge will use the award to extend his research programme on the environmental impact of cage culture of fishes to investigate the problems of South East Asia.

The Trustees of the Lady Tata Memorial Trust invite applications for Awards for research on leukaemia, in the Academic Year beginning 1 October 1983. The Trustees particularly wish to encourage studies of the leukaemogenic viruses in animals, the epidemiology, pathogenesis, and immunology of leukaemia.

Applications are invited for the 1983 Overseas Research Students Awards. These are granted by the Committee of Vice-Chancellors and Principals of the Universities of the United Kingdom. Around 600 Overseas Research Students Awards will be offered on a competitive basis which give partial remission of tuition fees to overseas postgraduate students. Full details of the application procedure are available from The Secretary, ORS Committee, Committee of Vice-Chancellors and Principals, 29 Tavistock Square, London WC1H 9EZ, UK.

The 1982 MacRobert Award, which comprises a Gold Medal and a cash prize of £25,000, has been made to **Professor Desty** of the BP Research Centre for the invention, development and sale of flare systems for disposing of surplus flammable gases.

Miscellaneous

From 1983, the journal *Metamedicine* published by D. Reidel Publishing Company of Dordrecht, Holland, will become *Theoretical Medicine*. The format of the journal will not change.

British Telecom will develop new degree courses at the Universities of Aston and York, UK for training 60 students to become electronic engineers specialized in telecommunications and computing. A grant of £100,000 a year for new equipment and additional teaching staff will be given by British Telecom. This support will be guaranteed for at least five years.

The British Royal College of Pathologists has established two qualifications for clinical cytogeneticists—the Diploma in Clinical Cytogenetics and Membership of the Royal College of Pathologists (Clinical Cytogenetics). Full details of these examinations and the qualifications required from candidates are available from Ms E.M. Fearn, Assistant College Secretary, The Royal College of Pathologists, 2 Carlton House Terrace, London SW1Y 5AF, UK.

ICI's Pharmaceuticals Division is donating £25,000 to establish a research fellowship at St Mary's Medical School, London University, UK. The fellowship will be dedicated to the late Alfred Spinks and will be advertised to attract the best young post-doctoral research workers in the field of drug metabolism.

The Wolfson Foundation has awarded a grant of £275,000 to the University of East Anglia, UK to provide accommodation for the Climatic Research Unit.

Meetings

9–10 February, **Conference on Cotton Dust Hazards**, Manchester (Mr J. Jackson, Shirley Institute, Manchester M20 8RX, UK).

17 February, **Other Plasmas**, London (The Meetings Office, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK).

21–23 February, **International Conference on Diet and Nutrition**, Tel Aviv (Dr C. Horwitz, Clinical Nutrition Unit, Department of Gastroenterology, Ichilov Hospital, 6 Weizman Street, Tel Aviv 64239, Israel).

21–25 February, **Course on Finite Elements for Mechanical Engineering Design**, Sheffield (Dr H.K. Kohler, Director for Engineering and Design Courses, Department of Mechanical Engineering, University of Sheffield, Mappin Street, Sheffield S1 3JD, UK).

21–25 February, **Laser Cutting, Welding and Materials Processing**, Colchester (The Liaison Officer, Laser Workshop Courses, University of Essex, Colchester CO4 3SQ, UK).

22 February, **Magnetic Filtration Meeting of the Filtration Society**, Manchester (The Hon. Secretary, The Filtration Society, c/o Department of Chemical Engineering, University of Technology, Loughborough, Leics. LE11 3TU, UK).

7–8 March, **Symposium on Cancers of the Rectum**, Paris (Mlle S. Hajeri, AFEC, 26 rue d'Ulm, 75231 Paris, Cedex 05, France).

7–11 March, **Principles of Optical Communications**, Colchester (The Liaison Officer, Laser Workshop Courses, University of Essex, Colchester CO4 3SQ, UK).

21–25 March, **Course on Basic Strain Gauge Application and Analysis**, Sheffield (Dr H.K. Kohler, Director for Engineering and Design Courses, University of Sheffield, Mappin Street, Sheffield S1 3JD, UK).

21–22 March, **Actions and Interactions of GABA and Benzodiazepines**, London (Dr N.G. Bowery, Department of Pharmacology, St Thomas's Hospital Medical School, London SE1 7EH, UK).

22–24 March, **Ergosterol Biosynthesis Inhibitors**, Reading (Dr D.R. Clifford, Long Ashton Research Station, Long Ashton, Bristol BS18 9AF, UK).

23–25 March, **Conference on Nuclear Structure and Particle Physics**, Liverpool (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK).

23–25 March, **Cell Biochemistry and Function**, Guildford (Ms J. Vare, The Conference Administrator, 1st International Meeting on Cell Biochemistry and Function, Butterworth Scientific Ltd, Journals Division, PO Box 63, Westbury House, Bury Street, Guildford GU2 5BH, UK).

4–8 April, **SPIE—The International Society for Optical Engineering's Technical Symposium East (Optics and Electro-Optics)**, Arlington (Ms S. Davis, Exhibit Manager, SPIE, PO Box 10, Bellingham, Washington 98227-0010 USA).

6–7 April **Annual Meeting of The National Council on Radiation Protection and Measurements**, Washington (W.R. Ney, National Council on Radiation Protection and Measurements, 7910 Woodmont Avenue, Suite 1016, Bethesda, Maryland 20814, USA).

11–13 April, **Spectroscopy and the Dynamics of Biological Systems**, London (Dr P.M. Bayley, National Institute for Medical Research, Mill Hill, London NW7 1AA, UK).

14 April, **Residual Stresses in Design and Fabrication**, London (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK).

18–22 April, **Course on Random Vibration**, Sheffield (Dr H.K. Kohler, Department of Mechanical Engineering, University of Sheffield, Mappin Street, Sheffield S1 3JD, UK).

19 April, **Symposium on Gastrointestinal Secretion — Mechanisms and Disorders**, London (Dr A.G. Butler, Glaxo Group Research Ltd, Ware, Herts SG12 0DJH, UK).

18 April, **The Neurobiology of Pain**, Leeds (Dr W. Winlow, Department of Physiology, University of Leeds, Leeds LS2 9NQ, UK).

26–28 April, **International Symposium on Capillary Chromatography**, Riva del Garda, Italy (Erba Science (UK) Ltd, Headlands Trading Estate, Swindon, Wiltshire, UK).

3–8 May, **Handicap '83**, Gothenburg (Svenska Massan Stiftelse, Box 5222, S-402 24 Goteborg, Sweden).

4–6 May, **Biotech '83**, London (Biotech '83, Online Conferences Ltd, Argyle House, Northwood Hills) HA6 1TS, Middlesex, UK).

9–13 May, **International Symposium on Remote Sensing of Environment**, Michigan (Remote Sensing Centre, Environmental Research Institute of Michigan, PO Box 8618, Ann Arbor, Michigan 48107, USA).

10–12 May, **Progress in Perinatal Medicine**, Florence (M.T. Zorza, Institute of Chemistry, School of Medicine, University of Brescia, Via Valsabbina, 19-25, 100 Brescia, Italy).

1–3 June, **Wind Energy Conference and Workshop**, Minneapolis (Dr Vas, FloWind Corporation, 21414 68th Avenue South, Kent WA 98031, USA).

30 May–3 June, **International Conference on Chromatographic Detectors**, Melbourne (The Secretary, International Conference on Chromatographic Detectors, University of Melbourne, Parkville, Victoria, Australia).

5–8 June, **International Symposium on Cell Differentiation and the Plasma Membrane**, Noordwijkerhout (C.A. Feltkamp, The Netherlands Cancer Institute, 121 Plesmanlaan, 1066 CX, Amsterdam, The Netherlands).

5–10 June, **International Congress of Pure and Applied Chemistry**, Cologne (Secretariat of the 29th IUPAC Congress, Dr W. Fritsche, c/o Gesellschaft Deutscher Chemiker, PO Box 900440, D-6000 Frankfurt/Main 90, FRG).

5–12 June, **Course in Advanced Immunology Regulatory Elements of the Immune System**, Beaufort, North Carolina (Dr D.W. Scott, AAI Course, Box 3010, Division of Immunology, Duke University Medical Centre, Durham, NC 27710, USA).

7–9 June, **International Conference on Satellite Systems for Mobile Communications and Navigation**, London (Conference Services Department, IEE, Savoy Place, London WC2R 0BL, UK).

7–11 June, **Wind River Conference on Genetic Exchange**, Colorado (Mr D.A. Morrison, Department of Biology, University of Illinois at Chicago, Box 4348, Chicago, IL 60680, USA).

12–16 June, **Congress of the International Society of Psychoneuroendocrinology**, New York (Professor F.L. Strand, Biology Department, New York University, Washington Square, New York, NY 1003, USA).

12–16 June, **International Catecholamine Symposium**, Goteborg (Fifth International CA Symposium, PO Box 33020, S-400 33 Goteborg, Sweden).

13–19 June, **International Congress on Mine Ventilation**, Harrogate (Conference Officer, The Institution of Mining and Metallurgy, 44 Portland Place, London W1N 4BR, UK).

17–18 June, **Symposium on Development of Visual Functions in Infants and Children**, Rotterdam (Dr J. van Hof-van Duin, Department of Physiology I, Erasmus Universiteit Rotterdam, PO Box 1738, 3000 DR Rotterdam, The Netherlands).

20–22 June, **International Symposium on Chromatography and Mass Spectrometry in Nutrition Science and Food Safety**, Montreux (Symposium Secretariat, c/o Italian Group for Mass Spectrometry in Biochemistry and Medicine, Via Eritrea, 62–20157 Milan, Italy).

17–24 June, **Congress of the International Seed Testing Association**, Ottawa (ISTA Secretariat, PO Box 412, 8046 Zurich, Switzerland).

20–24 June, **International Symposium on Cerebral Blood Flow and Metabolism**, Paris (Dr J. Seylaz, Scientific Officer, XIth CBF Symposium, Laboratoire de Physiologie et Physiopathologie Cerebrovasculaire, Faculte de Medecine, Universite de Paris VII, 10 avenue de Verdun 75010 Paris, France).